Ocular Biomarkers for Early Detection of Alzheimer's Disease

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Running Title: The eye and Alzheimer's disease.

Abstract

Alzheimer's disease (AD) is the most common form of dementia and is characterized clinically by a progressive decline in memory, learning and executive function and neuropathologically by the presence of cerebral amyloid deposits. Despite a century of research, there is still no cure or conclusive pre-mortem diagnosis for the disease. A number of symptom-modifying drugs for AD have been developed, but their efficacy is minimal and short-lived. AD cognitive symptoms arise only after significant, irreversible neural deterioration has occurred, hence there is an urgent need to detect AD early, before the onset of cognitive symptoms. An accurate, early diagnostic test for AD would enable current and future treatments to be more effective, as well as contribute to the development of new treatments.

While most AD related pathology occurs in the brain, the disease has also been reported to affect the eye, which is more accessible for imaging than the brain. AD-related proteins exist in the normal human eye and may produce ocular pathology in AD. There is some homology between the retinal and cerebral vasculatures and the retina also contains nerve cells and fibers that form a sensory extension of the brain. The eye is the only place in the body where vasculature or neural tissue is available for non-invasive optical imaging. This article presents a review of current literature on ocular morphology in AD and discusses the potential for an ocular based screening test for AD.

Keywords:

Retinal, vision disorders, cataract, lens, amyloid beta protein, aging, diagnosis

Contents

Alzheimer's Disease

Alzheimer's Disease (AD) is the most common form of dementia, affecting more than 26 million people worldwide [1]. The disease is characterized clinically by a progressive decline in memory, learning and executive function and neuropathologically by the presence of cerebral amyloid deposits. In addition to the debilitating symptoms endured by AD patients, the disease imposes a huge social and economic burden on society. AD is an incurable, degenerative and terminal disease usually diagnosed in people over 65 years of age [2]. It affects 5% of people aged 65 and 20–40% of those aged 85. The late-onset form of AD (LOAD) is the most common form of the disease and in the majority of cases, where there is no evidence of it being inherited, it is termed "sporadic AD". A rare form of AD, termed "early-onset familial AD" (EOFAD), is inherited in an autosomal dominant manner, and can occur in people as young as 30 years of age. Mild Cognitive Impairment (MCI) is considered a prodromal phase of AD, with 40-60% of people meeting criteria for MCI eventually progressing to AD, or about 5-25% per year [3].

AD is histopathologically characterized by a substantial loss of neurons in the brain, atrophy of the brain, as well as the deposition of extracellular amyloid β (Aβ) plaques and intracellular neurofibrillary tau tangles (NFT) [4]. The factors that may cause or accelerate the development of AD are not fully understood, and the exact contribution of plaques and tangles in causing symptoms of AD also remain to be established. General consensus in the field supports the "Amyloid Cascade Hypothesis", which states that an imbalance in Aβ metabolism in the brain is the fundamental cause of the neurodegeneration and cognitive decline in AD, though many studies suggest this effect of Aβ is at least partly mediated by increased tau phosphorylation. In recent years, much evidence has been gathered to show that several factors contribute to the risk of developing AD. These include diabetes, mid-life

obesity, and a history of heart disease or symptoms typically associated with heart disease such as high levels of low density lipoproteins (LDL) together with low levels of high density lipoproteins (HDL) [5].

The major protein component of the amyloid plaques is a peptide known as amyloid β (Aβ). Aβ peptides range from 39 to 43 amino acid residues in length. The longer (Aβ42 or Aβ43) peptides aggregate easily into fibrils, and small soluble oligomers of Aβ are believed to be a neurotoxic form of Aβ, whereas the large insoluble aggregates and plaques are relatively inert [6]. The Aβ peptide is proteolytically derived from its parent molecule, the amyloid-β protein precursor (AβPP). AβPP is an integral membrane protein that has been implicated in blood clotting [7] and as a regulator of neural plasticity and post-injury repair [8]. Aβ peptides are produced as a result of sequential cleavage of AβPP by enzymes known as the β-secretase (also known as β-site AβPP cleavage enzyme or BACE) and γ-secretase, respectively. The most common form is Aβ40, but it is the second most common form, Aβ42, which is more fibrillogenic and is thus associated with disease states [9].

Aβ has been shown to have a constrictive effect on the cerebral vasculature [10], and interestingly, to be neuroprotective at low physiological concentrations [11]. For this reason Aβ has been suggested to have a dual damage response role in the brain, by sealing the vasculature reducing brain oxygen requirement and combating oxidative stress [12]. A recent study also demonstrated that Aβ has significant antimicrobial ability against clinically relevant organisms, introducing an interesting hypothesis that infection might have a role in some forms of AD [13]. Whatever the role of Aβ in the healthy body, a large number of *in vitro* and *in vivo* AD studies, particularly those of EOFAD, have shown that higher than normal Aβ levels cause oxidative stress in the brain, resulting in synaptic loss, cell membrane damage, inflammation and ultimately result in neuronal cell death [14-15]. Such

studies have shown that EOFAD-associated mutations in the AβPP gene or the presenilin 1 and 2 genes (presenilin being an essential component of the γ-secretase enzyme) have been found either to increase total Aβ levels or to increase the production of Aβ42 [16-17]. Such increases in Aβ levels have been implicated in the pathogenesis of both familial and sporadic AD [14-15].

NFT are insoluble, twisted fibers of a protein called tau. Phosphorylated tau stabilizes the internal structure of healthy neurons, but in AD, tau proteins are hyperphosphorylated and tangle together. Again, recent evidence points to soluble tau rather than NFT as a cause of neuronal loss in AD [18-20]. When researchers turned off the mutant tau gene in a mouse model of AD, neurodegeneration was halted and cognitive performance improved even though NFT continued to accumulate [19]. While tau hyperphosphorylation is not specific to AD, there is strong evidence to indicate that it is essential for Aβ-induced cognitive decline to occur.

Treatments

At present there is no cure or disease modifying drug to effectively treat AD, but there are drugs that have proven to be beneficial at the level of reducing some symptoms for up to 18 months. There is an urgent need for effective treatments, with the number of cases worldwide forecast to exceed 100 million by 2050 [1]. Currently available drugs can delay or alleviate symptoms but do not slow the progression of the disease as they do not target the underlying cause. Pharmaceutical drugs currently available to treat the cognitive manifestations of AD include acetylcholinesterase inhibitors and an NMDA receptor antagonist. Research is continuing into drugs designed to prevent or reduce Aβ production,

to break up Aβ plaques or to prevent metal ion-Aβ interactions which may acccelerate Aβ aggregation [21-22]. For example, one suggested avenue of treatment involves modulating the activity of β and γ-secretases to produce mainly Aβ40 instead of the more harmful Aβ42 peptide. In other studies, immunizing transgenic AD mouse models with human Aβ42 peptide was found to prevent the build-up of Aβ plaques [23] which was associated with prevention of memory impairment, yet a successful outcome was not achieved in early clinical studies due to serious side-effects. However, immunotherapies that either prevent plaque deposition or enhance removal of plaques are still being actively investigated by pharmaceutical companies with a number of them in either phase II or III clinical trials. Anti-aggregation agents with the aim of removing plaques and/or preventing Aβ fragments from aggregating are also being examined [24] and are expected to enter clinical trials within the next 12 months.

In 2008, a discontinued Russian antihistamine drug *Dimebon (latrepiridine)* was found to stabilize cognitive and functional activity in AD patients [25]. However, this phase II study outcome has since been thrown into question by negative results from subsequent phase III trials [26]. Differences between the studies include mean age and cognitive performance of participants as well as the source of Dimebon. Further studies are now being conducted to determine the efficacy of Dimebon in conjunction with other drugs to treat AD. Dimebon inhibits cholinesterase and NMDA receptors and may enhance neuronal function and survival through its effects on mitochondria. Other non-pharmacological therapeutic approaches are currently being investigated. Of these, lifestyle modification, particularly physical activity, nutrition and mental stimulation are gaining considerable attention in the field. It has been demonstrated that a cognitively stimulating environment can reduce Aβ deposition in transgenic mice [27], and that increased cognitive activity in humans can

reduce the risk of AD [28]. A possible explanation for the mechanism by which cognitive stimulation might delay onset of AD has been identified [29]: researchers found that synaptic activity increases Aβ in local interstitial fluid. Cognitive stimulation transfers electrical activity away from brain regions with high 'default activity', hence reducing Aβ levels in these regions – particular regions which in fact end up with high levels of Aβ deposition in AD [30].

The above-mentioned treatments can be made available for AD patients only after they have been clinically diagnosed with the disease. Unfortunately AD is difficult to diagnose with absolute certainty. In addition, the cognitive symptoms on which diagnosis is based only become apparent after irreversible brain damage has already occurred. Hence the search for better AD treatments needs to be coupled with research into early detection of the disease.

Diagnosis

There is currently no definitive *pre-mortem* diagnosis for AD. Conclusive diagnosis of AD is only achieved following *post-mortem* examination of the brain for the presence plaques and tangles in the brain. A pre-mortem diagnosis of "probable AD" is currently made by clinical observations and testing of cognitive capacity and memory loss. Other dementias and conditions such as depression can have similar symptoms, thus confounding diagnosis [31]. A diagnostic error rate of about 10-15% has been reported for AD [32].

A diagnosis of probable AD is only possible when the condition has progressed and considerable neurological damage has already occurred. The increasing frequency of AD in the population, along with the need to treat the disease before cognitive symptoms arise,

calls for a sensitive and specific screening technology to identify high risk individuals before the brain is irreversibly damaged.

Biomarkers that have shown promise for the early detection of AD include a reduction in brain and specifically hippocampal volume (using MRI – magnetic resonance imaging), changes to brain function (using functional MRI) and changes in the concentrations of Aβ40, Aβ42 or tau in the cerebrospinal fluid (CSF). CSF biomarkers have proven to be the most accurate of the biomarkers investigated to date, with AD being shown to be associated with decreased A*β*42 levels and increased tau levels in the CSF [33]. However the latter procedure is invasive and patient compliance is anticipated to be low.

Another promising approach is PET imaging employing ligands, such as Pittsburgh Compound-B (PIB), which selectively bind to Aβ plaques *in vitro* and *in vivo,* enabling plaque load to be imaged in living patients [31-33]. Interestingly, many MCI and a significant percentage (30%) of cognitively normal elderly individuals show high brain retention of PIB and low Aβ42 levels in the CSF [34-35]. Further follow-up studies are required to determine if this identifies those individuals that will go on to develop AD, with one recent study supporting this hypothesis [36].

As mentioned earlier, in terms of genetic markers, certain mutations in AβPP, presenilin 1 and presenilin 2 are known to cause EOFAD [16-17]. One major genetic risk factor for sporadic AD has been known for some time, the apolipoprotein E (APOE) ε4 gene [37]. APOE ε4 has been implicated in modulating the metabolism and aggregation of Aβ [38]. Individuals with one copy of the APOE-ε4 allele have a 2-3 fold increased risk of developing AD by age 85 and those with two copies have a 12 fold increased risk compared to the general population [39].

Recent genetic association studies have revealed three more genes linked to AD, the Clusterin, PICALM and CR1 genes [40-41]. Also, a recent meta-analysis has found more than 20 genes that have a significant effect on AD risk [42]. However, while these genes may contribute to identifying high risk individuals, they are not sufficient on their own to make a diagnosis, nor is the major genetic risk factor, APOE ε4, which is associated with up to 50% of all AD cases. Hence genetic profiling is emerging as a technique for predicting the *risk* of an individual getting AD rather than providing diagnostic markers. It is possible that a panel of genetic and biological factors may together prove useful in determining high risk individuals.

Important properties of a biomarker for screening are sensitivity and specificity for the relevant disorder, as well as practicality of the biomarker measurement. Evaluation of the sensitivity and specificity of AD biomarkers involves comparison of biomarker measurements in AD cases and controls. Evaluation of potential AD biomarkers is thus hampered by the 10-15% diagnostic error rate in AD [32]. Studies incorporating post-mortem confirmation of AD can avoid this problem, but for most studies this is not possible, hence this diagnostic inaccuracy should be considered when interpreting results. A significant number of healthy controls (30%) may be high-risk clinically silent individuals, further confounding interpretation [43-44].

The combination of different biomarkers can increase sensitivity and specificity; combining CSF Aβ42 and tau parameters increases sensitivity and specificity above 80%. Indeed Simonsen *et al.* [45] found a panel of 5 CSF biomarkers for AD which when combined gave 100% sensitivity and 97% specificity on blinded independent data [45]. However, this study compared AD cases with healthy controls and hence doesn't provide information about the specificity of the test against other dementias or diseases. In terms of practicality, lumbar

punctures for CSF fluid samples are expensive, invasive and patient non-compliance is a major issue. Neuro-imaging (MRI, PET, CT) is also expensive and the number of facilities available for clinical use is not adequate for population screening. Therefore there is a need for new AD biomarkers that are more practical as well as more sensitive and specific. The absence of a suitable screening technology for AD has motivated some researchers to look for biomarkers that might exist elsewhere in the body, including the eye.

Vision in Alzheimer's Disease

Visual disturbance is often an early complaint of AD patients [46-47] and studies have reported reduced visual performance on tests of visual field [48-49], color vision [50-52], contrast sensitivity [53-55], backward masking [56-57], visual attention, motion perception, shape-from motion, visuo-spatial construction, visual memory [58-60], delayed saccadic initiation and movement and fixation problems [47, 61-63]. However, none of these deficiencies are specific to AD. The current literature is controversial and reflects the need for larger and more rigorous studies to be undertaken before the significance of this interesting approach for AD-screening can be conclusively evaluated.

Reported visual deficits in AD have generally been attributed to neuronal damage in the visual pathways of the brain [64-65] as well as deficiency of the neurotransmitter acetylcholine in AD, which is important in visual processing [66-68]. Indeed there is evidence that during the pathogenesis of AD, plaques and tangles occur in visual processing brain regions prior to their occurrence in the hippocampus [65]. Thus visual disturbance in AD may precede memory impairment. Since these visual deficiencies are not specific to AD, a newer field of research is investigating the hypothesis that there might be specific

pathological changes in the eye that accompany the disease. Such ocular changes may contribute to the visual deficiencies, or be a result of damage to the visual pathways of the brain. If the eye does harbor an endophenotype of AD, this would give hope for an ocular diagnosis of AD as well as opening up a new avenue for finding other genetic determinants of the disease. There is hope that the eye might yield biomarkers that are either highly specific for AD, or can contribute to an AD-specific risk-profile analysis in combination with genetic, cognitive and other tests. The following sections describe reported AD-associated changes to the eye.

Ocular Biomarkers for Early Detection of Alzheimer's Disease

Overview

Statistically significant ocular abnormalities that have been reported to accompany AD are tabulated below and discussed in the following sections;

(Table 1)

(Figure 1)

Pupil Responses in Alzheimer's Disease

The pupil is the aperture stop of the eye and controls the retinal illumination (see Figs. 1 and 2). The size of the pupil is regulated by the brain in response to the signals it receives from the eyes. Pupil size changes with brightness of incident light, emotions (e.g. fear), pain, cognitive tasks and with use of certain drugs (e.g. alcohol, opioids, LSD).

(Figure 2)

Pharmacological drugs delivered in eye-drops can affect the iris muscles, causing the pupil to contract or dilate, as is often required for ophthalmological examinations. A hypersensitive pupil response to a cholinergic agonist (*pilocarpine* - contraction) or antagonist (*tropicamide* - dilation) has been reported in AD patients [69-78]. The neurotransmitter acetylcholine is deficient in the AD brain [67, 79], hence cholinergic dysfunction in iris nerve cells is a possible explanation for the hypersensitive response, although in this case one would expect to find agonist hypersensitivity with antagonist subsensitivity, or vice versa. Alternative explanations more consistent with the dual hypersensitivity include AD related damage to the locus coeruleus brain region which is involved in pupillary control [80] or increased corneal penetration of the cholinergic eye-drops. One study using a fluorescent marker to evaluate corneal penetration of tropicamide found no difference between AD and controls [81], but further studies are required to confirm this result. It should also be noted that not all studies controlled for medications with anticholinergic effects.

The hypersensitive response was in some cases identified early in the disease progress, encouraging utilization for AD screening, however other studies have brought into question both the sensitivity and specificity of the test. Some studies found no significant hypersensitivity in AD [81-91], or a hypersensitive response in APOE-ε4 allele carriers rather than AD [78, 92], affecting carriers of this allele who are cognitively normal (although at increased risk of progressing to AD). A similar hypersensitivity has been reported in Down's syndrome subjects – who develop AD as a result of an extra copy of the AβPP gene [93], yet also in healthy young adults [87]. Still other studies have demonstrated modulation of the

pupil dilation response by eye color [93] or age [83-84, 94]. These results have left the reliability of the pupil dilation test for AD in question.

The pupil light reflex (Fig. 2) is the response of the pupil to a bright flash of light, involving rapid contraction followed by dilation back to original size. This reflex has been used as a neurological screening tool for disorders such as Parkinson's disease, Huntington's disease, schizophrenia, multiple sclerosis and trauma [95-98]. Since it is mainly a parasympathetic cholinergic response [99], the pupil light reflex could also possibly be affected if central cholinergic depletion in AD extends to the parasympathetic oculomotor system.

The pupil light reflex has been investigated as a non-invasive, ocular predictive marker for AD. Changes to a number of response parameters have been found in AD compared to healthy ageing [100], with a single parameter (reduced "maximum constriction acceleration") facilitating perfect classification in one study. This built upon previous studies which also found statistically significant differences in pupil flash response between AD and controls [91, 101-102], but Granholm *et al.* [91] found that AD and Parkinson's disease patients exhibited the same results. This suggests that specificity might also be an issue for this test, although "maximum constriction acceleration" was not considered in this study. The pupil flash response is also influenced by age [103]. More research is required to confirm the sensitivity and specificity of the pupil flash response parameters as a screening tool for AD.

The Ocular Lens and Vitreous Humor in Alzheimer's Disease

When light enters the eye it passes through the outer 'corneal' layer, followed by the aqueous humor and then the intra-ocular lens (see Fig. 1). The role of this anterior region of

the eye is to focus an optical image of the outside world onto the retina. One disorder that can disrupt this role is *cataract*; an opacification of the lens often due to protein aggregation (see Fig. 3). Cataracts are a common problem in the elderly, with progressive deposition of insoluble protein in the lens and extensive oxidative damage generally caused by environmental factors such as UV exposure [104-105].

The ability of the lens to focus light is achieved by its high protein concentration, higher than any other tissue of the human body. This high protein concentration, along with the optical accessibility of the lens, makes it ideal for the optical investigation of protein aggregation in disease, *in vivo*. The Aβ protein involved in the pathogenesis of AD in the brain has also been found to exist in the lens (Aβ40 and Aβ42), aqueous humor (Aβ40) and vitreous humor (Aβ42) of the normal human eye [106-107].

(Figure 3)

Remarkably, research indicates that a particular type of cataract (equatorial supranuclear cataract) might be specific to AD sufferers [106]. Slit-lamp microscopy of *ex-vivo* intra-ocular lenses of individuals with AD consistently revealed equatorial supranuclear cataracts. Subsequent histochemical analysis in the same study indicated that Aβ aggregates are present in the cytosol of the lens fiber cells co-localizing with the cataracts. This cataract has also been reported in Down's syndrome subjects [108] – who develop AD as a result of an extra copy of the AβPP gene.

These cataracts (or the initial Aβ aggregation in the lens) could thus be a biomarker for AD, although it is unknown at which stage of AD pathogenesis they occur. The location of the AD cataract is at the equatorial periphery of the lens posterior to the iris. The anatomical relationship of the lens relative to the iris renders AD-linked supranuclear opacification

virtually harmless with respect to visual impairment and difficult to detect on routine physical examination. However, these AD-linked lesions are readily observed by slit lamp ophthalmological evaluation in fully dilated subjects. If Aβ is indeed aggregating in the AD lens, leading to these cataracts, it is possible that the initial molecular changes could be detected non-invasively as an early screening or diagnostic test. Further research is needed to establish the specificity of these cataracts and lens Aβ aggregations to AD, since both AβPP and Aβ have been shown to increase in concentration in the normal mammalian lens in response to UV radiation or other oxidative effects [109].

For early diagnosis of AD, it has been proposed that a suitable eye-drop biomarker may enable Aβ in the anterior chamber to be stained and quantified [106]. Alternatively, the size of Aβ aggregates in the eye may facilitate non-invasive detection with optical scattering [110], spectral or autofluorescence techniques. Dynamic light scattering (DLS) uses back-scatter from a low energy laser beam to determine information about particle size, shape, movement and interactions. The technique is applicable to all eye tissues and has already shown promise for early cataract detection by monitoring the α–crystallin proteins that prevent protein aggregation in the lens [111-113].

Raman spectroscopy and AF techniques both involve illumination of the sample at a specific wavelength followed by measurement of the in-elastically scattered light at different wavelengths. Raman spectroscopy with principal components analysis has been used to distinguish AD from control *ex vivo* post-mortem brain tissues, based on spectra of protein aggregates [114-117]. AD brain tissues also exhibit visual and infrared-excited AF [116, 118]. These techniques could prove useful for non-invasive, early diagnosis of AD using the eye.

The eye contains three main fluid chambers called the anterior, posterior and vitreous chambers. The aqueous humor fills the anterior and posterior chambers (see Fig. 1),

providing nutrients to the lens and cornea and maintaining the convex curvature of the cornea. The vitreous humor has functional interactions with the lens and retina. No changes in these chambers have been reported in AD to date, but a change in Aβ42 and tau protein levels in the vitreous humor has been linked to retinal diseases such as diabetic retinopathy and "glaucoma concurrent with other ocular diseases" [107]. This change in protein levels is similar to that observed in the CSF in AD. Given the retinal degeneration observed in AD [119-123] and the recently reported common features between AD and glaucoma [61, 107, 121-130], the vitreous humor is an interesting focus for future research into ocular protein changes in AD.

The Retina and Optic Disc in Alzheimer's Disease

While the role of the anterior eye is to focus light onto the retina, the retina's task is to convert the light into electrical signals that enter the brain. The retina consists of multiple layers of neural and photoreceptor cells, along with nerve fibers and vasculature (see Figs. 4-6). The optic disc (or optic nerve head) is the interface between the retina and the optic nerve and is the location at which blood vessels and retinal nerve fibers leave the retina. Reduced visual performance in AD may be the result of pathology in the visual centers of the brain, but it also remains a possibility that retinal degeneration is involved, perhaps as a consequence of visual centre damage.

Ocular morphology reported in AD includes changes to the retinal vasculature and optic disc, retinal cell loss and thinning of the retinal nerve fiber layer (RNFL). A recent study by

Berisha *et al.* found that AD participants had a specific pattern of RNFL loss (measured by optical coherence tomography, OCT), narrower venules and decreased blood flow in these venules (both measured by a laser Doppler instrument) [120]. A limitation of the study was the small participant numbers (9 probable AD and 8 controls). It should also be noted that retinal vessel widths are influenced by age and race and can be altered in many disorders [131]. Decreased retinal venular caliber (vessel diameter), as reported in AD, has otherwise only been associated with high current blood pressure and low high-density lipoprotein (HDL) cholesterol levels [131]. In contrast, *increased* retinal venular caliber is associated with hyperglycaemia, obesity and inflammation [131].

(Figure 4)

If retinal vascular constriction is associated with AD, it is unclear whether the reduced blood flow might be responsible for the reported retinal cell death or instead might be a response to the associated reduction in metabolic demand. Aβ has been reported to exhibit a constrictive effect on cerebral vessels [10] but it is unclear whether Aβ levels are increased in the AD retina. Plaques and tangles have not been found in the human retina although Aβ has been isolated in aged human retinas [132]. Research on the retinas of AD transgenic mice has demonstrated Aβ plaques, hyperphosphorylated tau, increased microvascular deposition of Aβ and neuroinflammation [133-134]. Aβ immunotherapy in such transgenic mice has resulted in the clearance of retinal plaques but an increase in retinal amyloid angiopathy, identifying non-invasive retinal imaging as an alternative method for monitoring disease response to immunotherapy in these mice.

In addition to retinal venular constriction in human AD, Berisha *et al.* demonstrated significant thinning of the superior RNFL using optical coherence tomography (OCT, see Figs.

5 and 6). This region corresponds with the inferior visual field and these changes could explain the vision loss reported in this area in AD [48]. Other OCT studies have reported different patterns of RNFL loss (general, parapapillary and macular) [119, 135-136]. Iseri *et al.* found macular thinning in AD to be related to the severity of cognitive impairment [136]. Parisi *et al.* found RNFL thinning to be related to retinal dysfunction as revealed by abnormal pattern electroretinogram (PERG) responses [135]. Other studies have also found abnormal PERG responses in AD [137-138].

(Figure 5)

(Figure 6)

The loss of RNFL thickness in AD is linked to a depletion of retinal ganglion cells (RGC) and optic nerve axons as identified by histopathological studies [61, 139-143]. RGCs are the final common pathway that transfer visual information through the retinal nerve fibres and then the optic nerve into the brain (Fig. 7). A postmortem study by Blanks *et al.* [141] demonstrated a 25% decrease in RGC at the level of the foveal and parafoveal retina, while other studies have found no significant changes [144-145]. Further research is needed to confirm the RGC loss in AD, to establish any connection with Aβ in the retina and to confirm whether RGC loss is a cause of visual impairment in AD.

(Figure 7)

Retinal photography (Fig. 4) has also been used to identify RNFL abnormalities (nerve fiber loss) in AD [121-122], although one study indicated practical difficulties in using this approach for AD screening. The retinal nerve fibers are thick enough in the inner retina to make them visible in retinal photographs (Fig. 4), hence revealing areas of RNFL loss. While

Berisha *et al.* found reduced venular caliber in AD using a laser Doppler device, no retinal photography study has yet confirmed any retinal vascular changes in AD, possibly because such measurements using photography are complicated by issues of calibration and vessel boundary identification. Nevertheless, retinal photography does have the potential to detect RNFL and retinal vascular changes and hence needs to be pursued further in AD screening research.

AD is known to have a vascular component, with small-vessel disease, microinfarction and cerebral amyloid angiopathy (characterised by Aβ deposition in vessel walls) [146-147]. Aβ plaques as well as retinal microvascular deposition of Aβ have been identified in the retinas of AD transgenic mouse models [133] and could possibly be detected by non-invasive optical scattering [110], Raman spectroscopic or fluorescent tagging techniques. Given the homology between the retinal and cerebral microvasculatures [148], it is not unexpected that changes in the retinal vasculature might also occur in AD. Vascular topography, including the angles at which blood vessels bifurcate and the relationship between the widths of parent to daughter blood vessels at vascular junctions is optimized in healthy subjects in order to minimize shear stress across a vascular network [149-150]. Variations from the optimal geometrical topography are known to occur in particular vascular conditions [151-152]. Similar variations may occur in AD due to the disease's vascular component and hence are worthy of being explored with retinal photography.

Retinal photography and Scanning Laser Ophthalmoscopy (SLO) have both been used to demonstrate optic disc changes in AD, including optic disc pallor, pathologic disc cupping (hollowing-out), and thinning of the neuro-retinal rim [122-123]. Some of the ocular morphologies found in AD are also found in the eye disease glaucoma, specifically RNFL thinning, optic disc cupping and visual field loss. Glaucoma is second only to cataract as a

leading cause of blindness worldwide [153] and has ocular hypertension as its largest risk factor. The 5-fold higher chance of visual field defects and/or optic disc cupping found in AD has been interpreted as a higher occurrence rate of glaucoma in AD [124]. However, in this study, no AD participants had a family history of glaucoma, and ocular hypertension was not found in AD participants but was found in 7.5% of controls, reducing the likelihood that open-angle glaucoma was the cause. This is still in question though, with another study supporting the increased incidence of open-angle glaucoma in AD [154].

A greater than 10%/year decay in visual field and optic disc cupping were demonstrated in glaucoma patients who were later diagnosed with AD, whereas an average 3%/year decay in visual field was observed in glaucoma patients who did not develop AD, indicating that AD accelerates the progression of glaucoma symptoms [126]. However, increased rates of visual field defects and/or optic disc cupping have also been reported in Parkinson's disease [125] and since these changes are observed in the common eye disease glaucoma, they are unlikely to provide a test that has specificity for AD.

However, it is possible that investigations into these retinal changes in AD and glaucoma might yield interesting results about the pathogenesis of the diseases, as well as their treatment and monitoring. The similarities between the ocular effects of AD and glaucoma extend to changes observed in PERG recordings [127], the type of cells lost (large magnocellular RGC [61]) and possibly to the mechanism of RGC loss (apoptosis) [128-129]. In experimental glaucoma, Aβ co-localizes with RGC apoptosis and induces RGC apoptosis *in vivo* [130]. In addition, targeting the Aβ pathway with a β-secretase inhibitor, Congo red or Aβ-antibody has been found to be effective in treating experimental glaucoma (reducing RGC apoptosis) [130].

Chronic ocular hypertension (elevated intra-ocular pressure – IOP) has long been assumed to be a cause of RGC loss in glaucoma and has been shown to increase Aβ production in the rat retina [128]. Interestingly, in addition to its neuroprotective effects, a cholinesterase inhibitor used to treat AD has demonstrated dual therapeutic potential by reducing IOP in AD patients [155]. Current treatments for glaucoma are directed at reducing IOP, but evidence indicates that RGC loss still occurs in many glaucoma patients after successful IOP normalization, indicating that other mechanisms are involved.

In addition to glaucoma, Aβ has also been implicated in other retinal diseases such as age-related macular degeneration (AMD) [156-159]. This provides hope that these common neurodegenerative diseases (AD, Glaucoma and AMD) could be targeted simultaneously for treatment and monitoring, but reduces the likelihood of an Aβ-based, AD-specific biomarker in the retina. If Aβ changes in the retina are to be useful for AD screening, significant differences from the changes observed in retinal diseases must be identified. Aβ plaques as well as retinal microvascular deposition of Aβ have been identified in the retinas of AD transgenic mouse models [133]. If such changes occur in human AD, they could possibly be detected by non-invasive optical scattering [110], Raman spectroscopic or fluorescent tagging techniques. AD brain tissues exhibit visual and infrared excited auto-fluorescence (AF) [118]. Retinal AF changes are observed in a number of eye disorders including glaucoma, in which lipofuscin accumulation in parapapillary retinal pigment epithelial (RPE) cells causes AF changes [160]. Given the similarity of the retinal cell death process reported in glaucoma and AD [128-129], AF changes are also worthy of being investigated in the AD retina. It is hoped that these techniques could prove useful for non-invasive, early diagnosis of AD using the eye.

Conclusions

The terrible impact of AD, both on those directly affected and on society in general, creates a pressing need for better treatments. By the time a person is diagnosed with “probable AD” using current techniques, significant irreversible neuronal degeneration has already occurred. Therefore, research into better treatments must be paralleled by research into technologies to screen populations for AD, to identify cases before cognitive symptoms arise.

Ocular morphologies reported in AD give hope for a non-invasive, cost-effective screening test for AD. Evidence is accumulating in support of AD-related changes in the eye, but finding a sufficiently sensitive and specific ocular biomarker is proving to be a major challenge. Many reported ocular changes in AD also occur in other disorders. Optic disc changes, visual field defects and RNFL/retinal cell loss are also observed in the eye disease glaucoma. Retinal vessel widths are influenced by age and race and can be altered in many disorders. Similarly, pupil responses are influenced by age and eye color and are altered in many neurological disorders. Also, most studies into ocular morphology in AD have been limited by small participant numbers, few study groups and little relevant medical information on participants. Hence larger studies are required to confirm and investigate further these ocular changes in AD.

There is no doubt that the substantial diagnostic error rate in AD is confounding many aspects of AD research. A combination of new brain imaging techniques and CSF biomarkers has the potential to alleviate this problem, leading to more robust results in AD research, including ocular morphology. No studies have yet compared ocular morphology in AD to brain Aβ-loading or CSF protein changes, an approach that has the potential to shed light on

connections between ocular changes and AD. There are also many more ocular parameters left to investigate in AD.

Thus there remains scope for ocular changes to be utilised in AD screening or diagnostic purposes with greater sensitivity and specificity and at an earlier stage in the disease process. An ocular screening test for AD would benefit AD sufferers and researchers and possibly provide new insight into the molecular processes and genetic determinants of the disease. An ocular biomarker or biomarkers could turn out to be highly specific for AD, or to be a useful component in a multidisciplinary approach aimed at producing an earlier and more accurate diagnosis of Alzheimer's disease.

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Table 1: Reported Ocular Changes in AD

Part of the eye	Reported Ocular Changes in AD	Journal (Year)	Ref.	*N*(AD,Control)
Pupil	Enhanced pupil response to cholinergic drops	Science (1994)	[69]	19,32
		Neuroreport (1996)	[78]	25,24
		J Neurol Neurosurg Psych (1994)	[70]	26,23
		Neurobiol Aging (2003)	[72]	14,30
		Acta Neurol Scand (1996)	[73]	24,50
		Neuropsychobiology (1999)	[74]	29,29
		Biol Psychiatry (1997)	[75]	67,80
		Rev Neurol (1996)	[76]	10,20
		Nippon Ronen Igakkai Zasshi (1996)	[77]	53,29
Pupil	Altered pupil flash response	Aging Clin Exp Res (2007)	[100]	23,23
		Int J Psychophysiol (2000)	[101]	10,5
		Int J Psychophysiol (2003)	[91]	15,30
		J Neurol Neurosurg Psych (1997)	[102]	9,9
Lens	Aggregation of Aβ, Supra-nuclear cataract	Lancet (2003)	[106]	9,8
Retina	Narrow retinal veins and decreased venular blood flow	Invest Ophthalmol Vis Sci (2007)	[120]	9,8
Retina	Retinal Nerve Fiber Layer (RNFL) thinning	Invest Ophthalmol Vis Sci (2007)	[120]	9,8
		Neurosci Lett (2007)	[119]	26,38
Retina	RNFL abnormalities and cell loss	Acta Neurol Scand (1996)	[121]	26,23
		Archives of Ophthalmology (1991)	[122]	26,30
		Neurology (2006)	[123]	40,50
Retina	Abnormal pattern electroretinogram (PERG)	Ann Neurol (1989)	[137]	6,6
		Ann Neurol (1989)	[138]	13,30
Optic Disc	Optic disc pallor, pathologic disc cupping, and thinning of the neuro-retinal rim	Acta Neurol Scand (1996)	[121]	26,23
		Archives of Ophthalmology (1991)	[122]	30,32
		Neurology (2006)	[123]	40,50

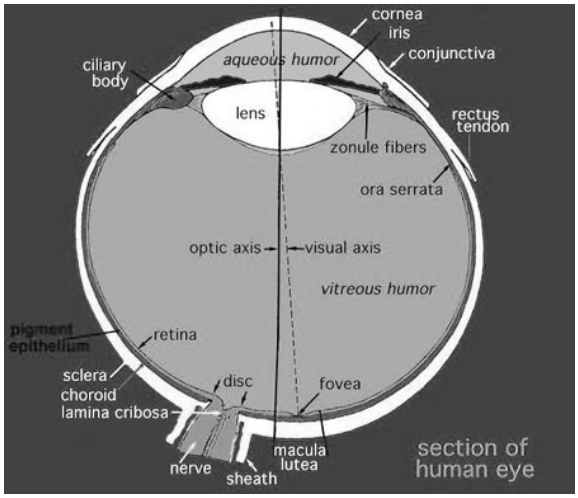
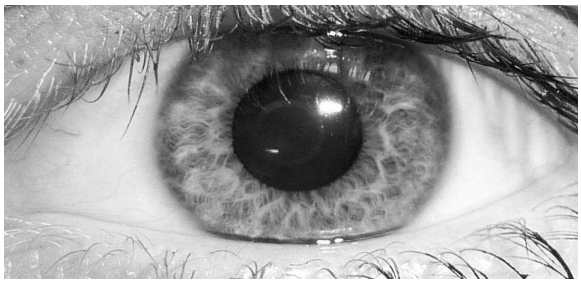
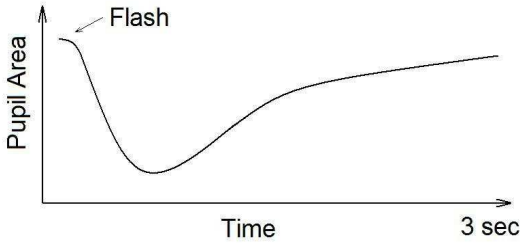


Figure 1: Cross-section of the human eye





(a) (b)

Figure 2. (a) The human eye. The pupil is the central transparent aperture (appearing as black), surrounded by the iris. (b) The graph of a pupil flash response, showing the pupil contraction resulting from a bright flash of white light.

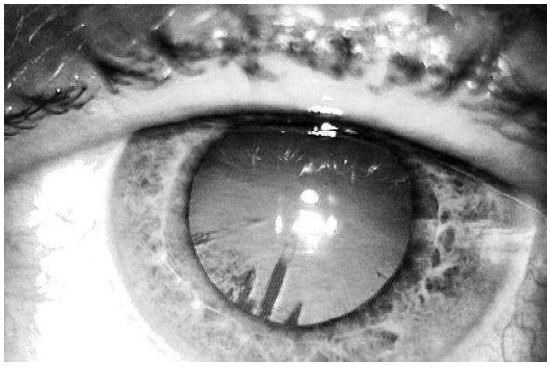


Figure 3. Retro-illumination photograph of a lens with cataract, pupil dilated with tropicamide eye drops. A different type of cataract has been linked with Alzheimer's disease.

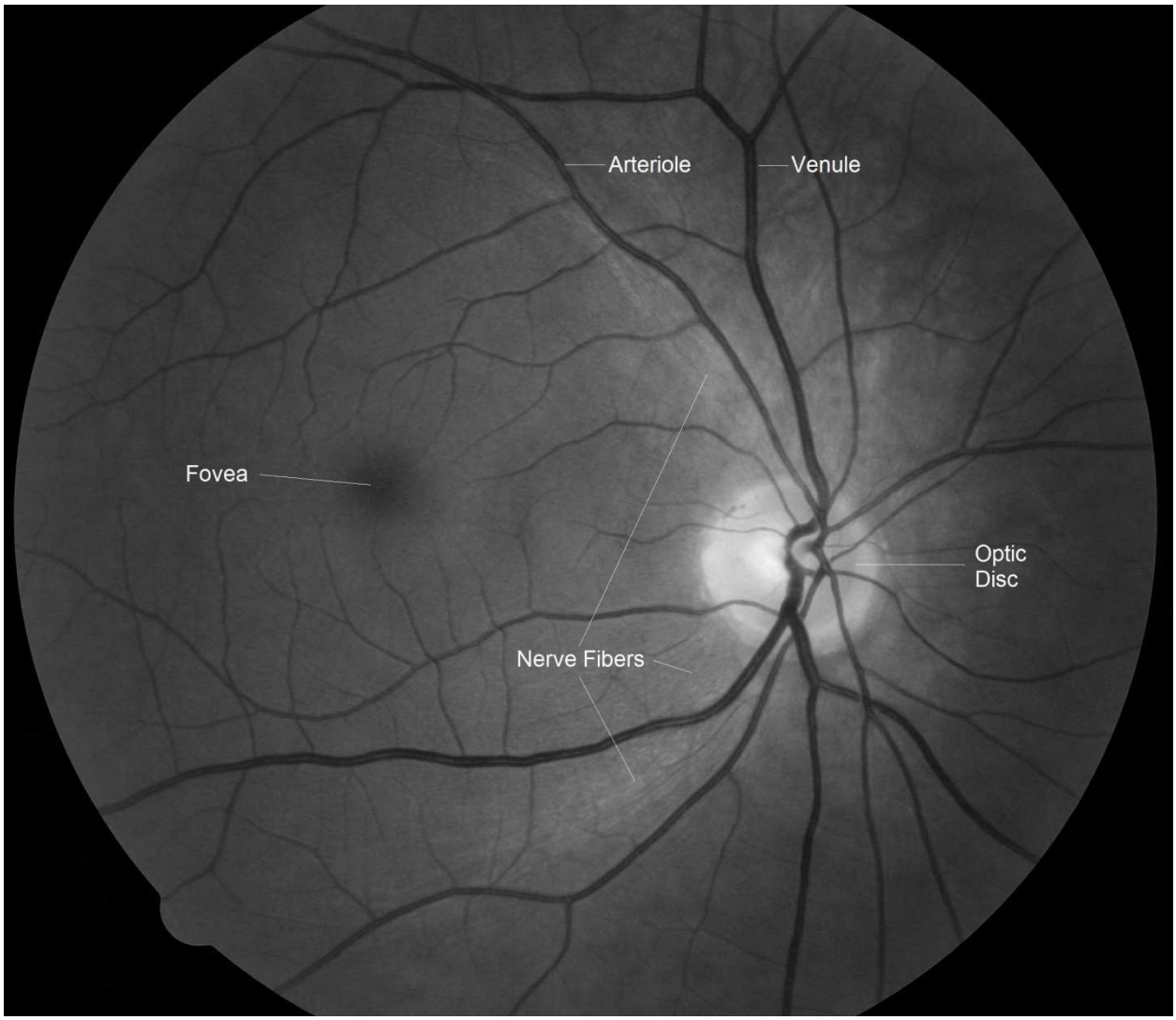


Figure 4. Digital retinal photograph displaying the optic disc in centre, with retinal arterioles and venules (darker) and lightly opaque retinal nerve fibers coursing to the optic disc.

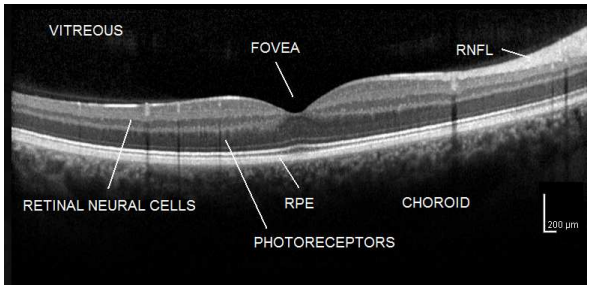


Figure 5. OCT scan showing the retinal layers around the fovea. The layer closest to the vitreous humour is the retinal nerve fiber layer (RNFL) which contains fibers emerging from the retinal ganglion cells below. Also just beneath the RNFL is the retinal vasculature (evident from the vertical shadows cast in this OCT scan). Beneath the retinal ganglion cells are the bipolar, amacrine and horizontal cells, followed by a layer of photoreceptor cells. The photoreceptor cells are nourished by the deeper retinal pigment epithelium and a rich posterior vascular layer called the choroid. *OCT scan courtesy of Chris Barry, Lions Eye Institute, Perth, Australia.*

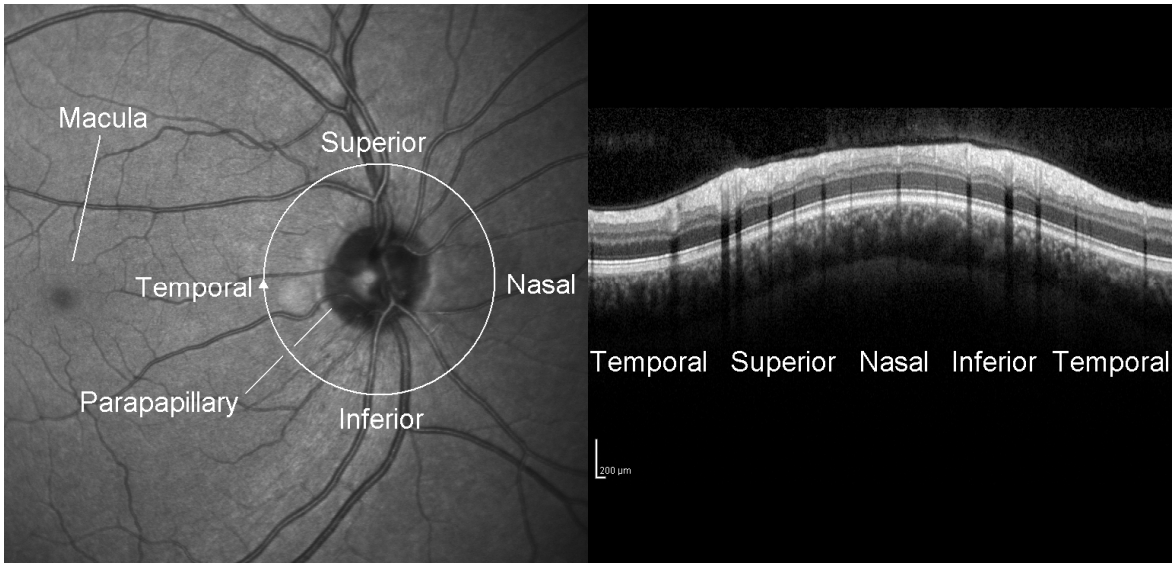


Figure 6. OCT scan circling the optic disc. The image on the right shows the retinal layers detected in an OCT scan traversing a circular path around the optic disc, as illustrated in the retinal photograph on the left. The RNFL is thickest in the superior and inferior quadrants. RNFL studies in AD have had varying results, indicating superior, general, macular or parapapillary thinning. *OCT scan courtesy of Chris Barry, Lions Eye Institute, Perth, Australia.*

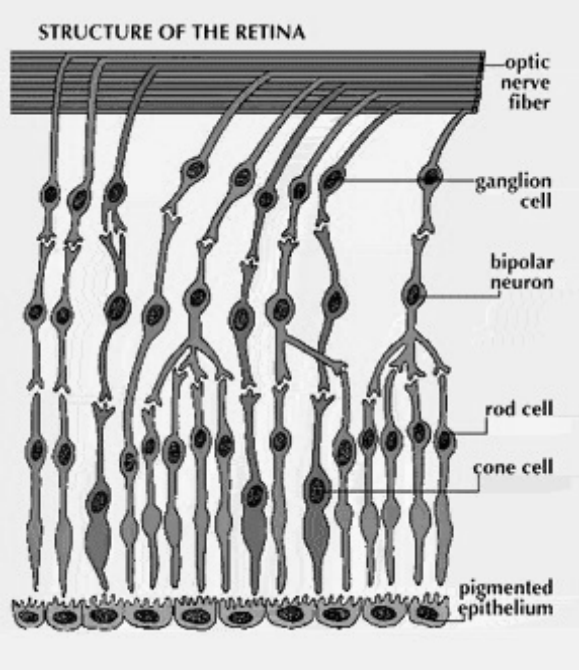


Figure 7: Layers of cells in the retina. Light must pass through the retinal nerve fiber layer and retinal neural cells (ganglion, bipolar, etc.) before reaching the photoreceptor cells (rods and cones).