Retinal changes in Alzheimer’s disease—integrated prospects of imaging, functional and molecular advances

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ABSTRACT

Alzheimer’s Disease (AD) is a devastating neurodegenerative disorder of the brain, clinically characterised by cognitive deficits that gradually worsen over time. There is, at present, no established cure, or disease-modifying treatments for AD. As life expectancy increases globally, the number of individuals suffering from the disease is projected to increase substantially. Cumulative evidence indicates that AD neuropathological process is initiated several years, if not decades, before clinical signs are evident in patients, and diagnosis made. While several imaging, cognitive, CSF and blood-based biomarkers have been proposed for the early detection of AD; their sensitivity and specificity in the symptomatic stages is highly variable and it is difficult to justify their use in even earlier, pre-clinical stages of the disease. Research has identified potentially measurable functional, structural, metabolic and vascular changes in the retina during early stages of AD. Retina offers a distinctively accessible region and allows attempts to monitor biochemical changes and protein aggregation pathology in the retina in AD. This review summarises the existing knowledge that informs our understanding of the impact of AD on the retina and highlights some of the gaps that need to be addressed. Future research will integrate molecular imaging innovation with functional and structural changes to enhance our knowledge of the AD pathophysiological mechanisms and establish the utility of monitoring retinal changes as a potential biomarker for AD.

1. Introduction

Alzheimer’s disease (AD) is a chronic and multifactorial neurodegenerative disorder characterized by progressive cognitive impairments, behavioral abnormalities and disturbances in circadian rhythm (Ismail et al., 2016). The disease also involves several less frequent clinical presentations that are gradually being established. The
clinical and molecular features of AD were initially reported by German scientist Aloisius Alzheimer in 1906 and the disease named in his honour by his colleague and mentor, Emil Kraepelin (Toodyan, 2016). The disease presently accounts for about 70% of all dementia cases globally. Age is the major risk factor and the probability of a person doubling approximately every 5 years after the age of 65 (Corrada et al., 2010). Therefore, as the global proportion of older people increases, the disease has emerged as one of the largest medical and socio-economic problems of our time. Since the 1990s, the prevalence of dementia has doubled, and approximately 50 million people are believed to be currently living with this disorder as continually, the retina is an extension of the CNS and is composed of several populations. It is protected by blood-retinal barrier (BRB) that implements selective blood-retinal permeability resemblant to that of the blood-brain barrier (BBB). The eye also exhibits immune response processes commensurate with that observed in the brain and spinal cord. Thus, as the retina provides a "strategic glimpse of the brain", retinal examination can be a novel, non-invasive, inexpensive, and tractable marker for AD diagnosis. Various retinal changes in AD have been investigated in the last three decades, and here we provide a brief examination can be a novel, non-invasive, inexpensive, and tractable marker for AD diagnosis. Various retinal changes in AD have been investigated in the last three decades, and here we provide a brief

| Abbreviations | Clinical features |
|---------------|-----------------|
| ADAS-Cog      | AD assessment scale-cognitive subscale |
| ANX776        | annexin-5 labelled with fluorescent dye |
| ApoE          | apolipoprotein E |
| APP           | amyloid precursor protein |
| AP1S2         | Adaptor Related Protein Complex 1 Subunit Sigma 2 |
| Aβ            | amyloid beta |
| BACE1         | Beta-secretase 1 |
| C1q           | complement component 1q |
| BMO-MRW       | Bruch’s membrane opening−minimum rim width |
| CDR           | clinical dementia rating |
| CHAT          | Choline O-acetyltransferase |
| CHOP          | C/EBP homologous protein |
| CRVE/CRAE     | central retinal artery and vein equivalent |
| CSTF2         | cleavage stimulation factor subunit 2 |
| CSV           | contrast sensitivity vision |
| cTORTa        | curvature tortuosity of the arterioles |
| DHX38         | DEAH-Box Helicase 38 |
| DVA           | dynamic vessel analyzer |
| Eefla1        | Eukaryotic Translation Elongation Factor 1 Alpha 1 |
| Eefle1        | Eukaryotic Translation Elongation Factor 1 Epsilon 1 |
| elf2          | E74-like factor |
| elf4          | ETS-related transcription factor |
| ERG           | electroretinogram |
| ESR1          | Estrogen Receptor 1 |
| F4/80         | EGF-like module-containing mucin-like hormone receptor-like 1 |
| FAZ           | foveal avascular zone surface |
| FDa           | fractal dimension |
| GCC           | Ganglion cell complex |
| GCL           | ganglion cell layer |
| GFAP          | glial fibrillary acidic protein |
| GSK3β         | glycogen synthase kinase 3β |
| HSI           | hyperspectral imaging |
| IL            | interleukin |
| INL           | inner nuclear layer |
| LGN           | lateral geniculate nucleus |
| LRPI-1        | low density lipoprotein receptor-related protein 1 |
| MAPT          | Microtubule Associated Protein Tau |
| mRGC          | melanopsin ganglion cells |
| MRI           | magnetic resonance imaging |
| Mrp12         | Mitochondrial Ribosomal Protein L2 |
| MS            | multiple sclerosis |
| MRPL2         | 39S ribosomal protein L2 |
| mTOR          | mammalian target of rapamycin |
| NFL           | Nerve fiber layer |
| NFT           | neurofibrillary tangles |
| NFkB          | nuclear factor kappa-light-chain-enhancer of activated B cells |
| OCT           | optical coherence tomography |
| OCTA          | optical coherence tomography angiography |
| opa1          | OPA1 Mitochondrial Dynamin Like GTPase |
| OPN           | osteopontin |
| p70S6K        | Ribosomal protein S6 kinase beta-1 |
| PD            | Parkinson’s disease |
| PDGFβ         | platelet-derived growth factor receptor beta |
| pERG          | pattern electroretinogram |
| PHF           | paired helical filament |
| PI            | propidium iodide; |
| POAG          | primary open angle glaucoma |
| pRNFL         | Peripapillary retinal nerve fiber layer |
| PSEN1         | presenilin-1 |
| PSD-95        | Postsynaptic density protein 95 |
| Psma5         | Proteasome 20S Subunit Alpha 5 |
| Psmb2         | Proteasome 20S Subunit Beta 2 |
| Psmd3         | 26S proteasome non-ATPase regulatory subunit 3 |
| pSTR          | positive scotopic threshold response |
| pTau          | phosphorylated tau |
| Rbmx1         | RNA binding motif protein, X-linked-like-1 |
| SC            | superior colliculus |
| SUVr          | standardized uptake value ratio |
| TREM2         | triggering receptor expressed on myeloid cells 2 |
| Rp9           | Retinitis pigmentosa 9 |
| Rp135a        | Ribosomal Protein L35a |
| R9136a        | Ribosomal Protein L36a |
| SNAP-25       | Synaptosomal-Associated Protein |
| TrkB          | tropomyosin receptor kinase B |
| TUNEL         | terminal deoxynucleotidyl transferase dUTP nick end labelling |
| TUJ 1         | β-Tubulin 3 |
| VEP           | visual evoked potential |
| Zn-DPA        | Bis(zinc(II)-dipicolylamine) |
historical timeline of the key studies that have propelled research into this space, as shown in Fig. 1.

AD most likely begins with the accumulation and propagation of misfolded Aβ assemblies in the brain, followed by the hyper-phosphorylation of tau protein leading to paired helical filaments (PHFs) and neurofibrillary tangles (NFTs) (Braak and Braak, 1991; Spires-Jones and Hyman, 2014). These molecular events are accompanied by sequelae of neurodegenerative changes and glial cell activation in the brain (Andreone et al., 2020; Itagaki et al., 1989). Currently, a diagnosis of AD is based on clinical symptomatology combined with biomarker evidence of AD neuropathological change (McKhann et al., 2011; Sperling et al., 2011). With continuous imaging and CSF biomarker developments, an organized approach was required to bring uniformity in research and diagnostic criteria for AD. Towards this end, National Institute of Neurological Disorders and Stroke (NINDS, USA) and Alzheimer’s Association (USA) working group in (2007) recommended neuropsychological assessment as a means to establish cognitive impairment in patients and diagnose them into possible or probable AD cases (Cummings, 2012; Dubois et al., 2007). The AD diagnostic criteria was refined by National Institute on Aging and Alzheimer’s Association (NIA-AA) in 2011 and again revised in 2018 wherein an understanding was reached with an objective to define and stage AD spectrum for

![Fig. 1. Timeline marking various reports with a focus on elucidating retinal changes in AD.](image-url)
Based on these recommendations, AD is defined to include cases in which there is a combined clinical evidence for Aβ (A), pathological tau (T) and neural injury (N) biomarkers ([AT(N)]. Complete biomarker investigations based on AT(N) recommendations can be contingent to either imaging or CSF analytical approaches or combination of both strategies (Jack et al., 2018). Magnetic resonance imaging (MRI), computerized tomography (CT), and positron emission tomography (PET) scans are frequently used to image the build-up of amyloid and accompanying cerebral atrophy in the brain in cases where AD is likely (Thientunyakit et al., 2020). Since Robertson and colleagues (NY, USA) designed initial PET instrumentation in 1961, the performance and

Fig. 2. Schematic representation illustrating various approaches that can be used to detect and monitor AD changes using the visual system. The anatomical relationship of the eye and the brain is presented. Various retinal and brain tests that can potentially differentiate healthy subjects from AD are presented and labelled. fNIRS: Functional near infrared spectroscopy, MEG: Magnetoencephalography, TMS: Transcranial magnetic stimulation, pERG: pattern ERG, OCTA: optical coherence tomography angiography.
scope of the technique has greatly expanded in AD diagnosis (Lu, 2019). This is particularly made possible by development of PET tracers that selectively bind Aβ and tau proteins and distinguish metabolic changes in the brain (Hanseeuw et al., 2019). Amyloid and Tau-PET are very disease specific that measure the exact pathological process but with a certain threshold and limited spatial resolution. MRI also has limitations in terms of spatial resolution. Cerebrospinal fluid (CSF) provides a good reflection of brain processes, although collecting it requires lumbar puncture, which hampers widespread acceptability as a first-line test to screen AD asymptomatic cases. The validated AD biomarker assays for Aβ and phosphorylated tau (pTau) changes in CSF, can enable differential diagnosis of AD (Gordon et al., 2016; Villemagne et al., 2018). PET imaging also is not easily translatable to population-wide screening mainly due to high costs involved, low half-life of radioligands such as fluorodeoxyglucose (110 min for F-18 FDG) and exposure to ionizing radiations. Consequently, accurate prediction of early, non-symptomatic stages of AD, even with these gold-standard tests is highly variable. Monitoring retinal changes may thus provide a cost effective and patient friendly way to diagnose AD. More recently, plasma p-Tau 181 is reported to track with tau-PET scores and CSF levels of p-tau181, establishing it as a potential tool for screening of MCI and asymptomatic AD patients (Janelidze et al., 2020; Thijssen et al., 2020). Development of early stage specific blood biomarkers is likely to greatly improve the AD diagnosis.

Various studies have increasingly established that the retina is affected in patients with AD (Golzan et al., 2017; Grimaldi et al., 2019; Hadoux et al., 2019; London et al., 2013; Shi et al., 2020). Since the first reports of degenerative changes in the optic nerve in AD patients (Cogan, 1985; Hinton et al., 1986; Sadun et al., 1987), retinal pathology in AD has been specifically reported as the loss of RGCs and the NFL, the deterioration of the macular ganglion cell complex, and optic nerve axonal diminution (Cheung et al., 2015; Danesh-Meyer et al., 2006; Gao et al., 2015b; Gupta et al., 2016b; Kesler et al., 2011; Koronyo-Hamaoui et al., 2011; Parisi et al., 2001). Studies from our team and others have also described retinal blood flow and vascular alterations (Cheung et al., 2015; Golzan et al., 2017), astroglial and inflammatory changes (Grimaldi et al., 2018) associated with the AD pathophysiology (Cheung et al., 2015; Feke et al., 2015; Gao et al., 2015b; Krasodomska et al., 2010; Ramirez et al., 2017). A schematic representation of the established and emerging visual system examination technologies is shown in Fig. 2, and exemplifies that when integrated with brain imaging, biomarker and neuropsychological assessment, the eye could potentially improve AD risk assessment, detection, and monitoring.

The inability to diagnose AD early or accurately monitor the progression of the disease in a viable and non-invasive way is a major impediment in both disease management and therapeutic development (McAleese et al., 2016). Of late, several strategies including drugs and lifestyle-based changes have been proposed to be protective in cases of AD. However, most clinical trials have not been able to meet their primary objectives (Egan et al., 2019; Honig et al., 2018) with an exception of Aducanumab (Biogen) which was able to meet pre-defined end points when administered at a high dose (Schneider, 2020). One potential explanation for these unremarkable outcomes could be the current technological limitations in monitoring subtle brain changes due to inherent difficulties in accessing the intracerebral pathways. Another possibility underlying this failure could be that biochemical alterations are underway in the brain nearly 10–20 years before the development of the clinical syndrome. Consequently, it is vital—clinically ascertain AD pathology at earlier stages— when synaptic connections and neuronal functions may still be intact (Blinnemo et al., 2015; Jack et al., 2018). Recent evidence, obtained from both human and animal studies, continues to reinforce the feasibility of retinal examination to detect AD symptoms (Nguyen et al., 2017). The remainder of this review elucidates the use of recent advances in retinal imaging and its functional and structural determinants as novel biomarkers for AD. We also comprehensively summarize data regarding molecular changes in the AD retina to understand disease mechanisms. The ongoing clinical trials, future implications and overlap with other retinal neurodegenerative disorders is also discussed.

2. Amyloid and tau protein changes in the retina

2.1. Neuropathological features of amyloid in the retina

Clinical, post-mortem, and animal model studies have in general, revealed the association of AD pathophysiology with retinal dysfunction and structural alterations. Genetic, electrophysiological, and biochemical evidence suggests that build-up of Aβ peptides in the brain is one of the earliest events in AD pathology, which ensues from an imbalance between its production and clearance (Selkoe and Hardy, 2016). Cortical levels of Aβ are increased with low Aβ42 in CSF and these changes can be assessed using amyloid imaging and CSF analysis as per AT(N) scheme (Jack et al., 2018). Aβ is small peptide fragment of 36–43 amino acids (4–5 kDa), that is derived from transmembrane amyloid precursor protein (APP) (Marsden et al., 2011). APP is expressed as several isoforms ranging in size from 695 to 770 amino acids (100–140 kDa), although APP695 is the one which is predominantly expressed in neurons (Chen et al., 2017). Aβ peptides aggregate into soluble oligomers and then into transitional globular structures, and are believed to initiate a cascade of secondary pathologies involving distinct inflammatory responses, chronic vascular compliance issues, oxidative stress, mitochondrial dysregulation, and progressive synaptic and neuronal loss (Jack et al., 2018; Polanco et al., 2018; Selkoe and Hardy, 2016). Additionally, compromised glial cell capacity to mediate Aβ uptake has been shown to play a vital role in AD pathogenesis, and thus modulating glial cell function has been suggested as a potential strategy to reduce the burden of Aβ. Both APP and pTau are hallmark proteins of AD pathology, and a growing body of evidence indicates alterations in these proteins in the retinal tissues of both animal models of AD and human post-mortem samples. This is based on enhanced amyloid deposits and phosphorylated tau reactivity reported in several studies while others have highlighted detection of intracellular APP positivity in human AD postmortem retinal tissues, without evident Aβ plaque formation (Figs. 3 and 4). This was established by using multiple APP and Aβ specific antibody stainings in the superior and medial regions of the human AD retinas (den Haan et al., 2018c; Ho et al., 2014; Williams et al., 2017).

Most cases of AD are sporadic that may be linked to complex interactions between multiple genes and epigenetic factors while about 3% cases are attributed to genetic mutations (Selkoe, 2011). Animal models of both sporadic AD such as degus, beagle dog etc. and transgenic models that specifically modulate APP, tau (MAPT), neuroinflammation genes leading to protein over-expression and aggregation pathology are known (Prpar Mihevc and Majdic, 2019; Sasaguri et al., 2017). These genetic models offer a more tractable system for AD research but have limitations in deducing correlation of pathological mechanisms with human sporadic AD condition. Aβ deposition in the retina has been detected in transgenic drosophila, common degu and in the transgenic mice Tg2576, 3xTg-AD, APPSWE/PS1ΔE9, and APPSWE/PS1M146L/L286V, 5xFAD etc. and tau pathology reported in mutant MAPT, APP/PS1/MAPT models at different ages (Chiaus et al., 2017; Du et al., 2015; Gupta et al., 2016b; Liu et al., 2009).

Our group and other researchers have identified Aβ accumulation and assemblies of mini-deposits in the retina, mainly in the GCL and NFL, of APP/PS1 and 3xTg-AD mice as early as 2–3 months of age, which is several months prior to when significant Aβ deposition is known to occur in the hippocampus and cerebral tissues (Koronyo-Hamaoui et al., 2011; Mirzaei et al., 2019b). Enhanced Aβ deposition has also been demonstrated in retinal flat-mounts of ageing APP/PS1 mice (13–16 month), suggesting that sustained upregulation of soluble Aβ could gradually lead to deposit formation in the retina (Gupta et al., 2016b). Immunoblotting analyses have also shown that soluble Aβ and its various oligomers are indeed elevated in APP/PS1 mouse retinal tissues
influence. Variations in light exposure, are some of the factors that could further blood-vessel diameter, choroidal changes, tissue permeability, and APP/PS1 mice models (Dutescu et al., 2009). These findings correlate with observed increases in β-Aβ deposition in the two tissues. Another recent study also quantified Aβ oligomers in 5xFAD mouse model of AD using immunohistochemical analysis. It was observed that oligomer levels peaked at around 6 months of age, before gradually declining as the animals progressed to beyond one year of age. This was consistent with the recruitment of soluble oligomers into complex insoluble diffused structures leading to increased retinal amyloid deposition with age (Habiba, 2020). A recent study revealed that the Aβ1-40 species is particularly enriched in the inner layers of the central region of the human postmortem retina (Shi et al., 2020). Evidence of increased Aβ deposits in retinal regions that are particularly rich in rod cells, means that these could influence contrast sensitivity and visual motion perception changes observed in AD subjects (Koronyo et al., 2017; Risacher et al., 2013). Changes in retinal blood flow and blood-vessel diameter, choroidal changes, tissue permeability, and variations in light exposure, are some of the factors that could further influence Aβ aggregation or its clearance from specific regions of the retina. Aβ deposition in AD patient retinas was further shown to correspond to the loss of melanopsin-containing RGCs, the cells of which have been shown to play an important role in regulating circadian rhythm (La Morgia et al., 2016). This selective loss of photosensitive RGCs could decode the mechanistic basis of circadian sleep-cycle disruption and visual field defects that underlie AD, even in the early stages (Ju et al., 2013). The retinal Aβ pathology and the unique pattern of its deposition has also been shown to occur concomitantly with brain amyloid pathology (Hadoux et al., 2019; Koronyo et al., 2017).

Apart from changes in the neurosensory and vascular retina, some studies have also documented the detection of Aβ peptides and protein aggregates in other ocular regions, such as the lens, vitreous humor, corneal epithelium and choroidal tissues in human and animal studies (Dong et al., 2018; Goldstein et al., 2003). Investigating these changes along with tear film analysis has been gaining traction, however larger sample sizes will be required to obtain meaningful results. A similar deposition of Aβ in the lens has been observed in cases of Down’s syndrome (Moncaster et al., 2010). Down’s syndrome patients possess three copies of APP gene which results in Aβ over-production, and studying molecular pathological changes in these patients could help understand the effects of Aβ on the eyes (Moncaster et al., 2010). The structural and functional ramifications of Aβ aggregation in the retina in AD, as well as in other neurodegenerative disorders of the retina and eye, are currently poorly understood. However, the deleterious effects of Aβ—including protracted synaptic transmission, dendritic network remodeling of cholinergic amacrine cells, and inadequate levels of acetylcholine—are believed to be some of the key pathological changes related to AD (Reed et al., 2017). While these ancillary indicators are shared by several other neurodegenerative diseases, these molecular clues could reflect the advancement of AD and aid-in clinical stratification of the disease.

2.2. Neuropathological features of tau in retina

In addition to amyloid deposition, AD pathology typically involves increased amounts of pTau in neurophil filaments, neurofibrillary tangles, and neuritic plaques, primarily along cerebral cortical stretches (Brottscheider et al., 2015; Gibbons et al., 2019). Tau is a heterogenous...
group of microtubule-associated proteins derived through alternative splicing of MAPT and is abundantly expressed in the neurons in CNS. The protein isoforms range in length from 352 to 441 residues, with 79 Ser and Thr sites that can potentially undergo phosphorylation by Erk, GSK3 and CDK5, out of which approximately 38 phosphorylated sites have previously been reported in AD patients (Hanger et al., 2007; Martin et al., 2013). The hyperphosphorylation of tau proteins reduces their ability to interact with and bind to microtubular proteins (Lathuiliere et al., 2017). Increased cortical tau as assessed by tau-PET and enhanced p-Tau levels in CSF can be useful AD biomarkers as per AT(N) scheme. CSF total tau level is also one of the biomarkers for neurodegeneration in AD brain (Jack et al., 2018).

Within the retina, the tau protein is expressed in layers enriched with axonal connections, such as the inner and outer plexiform layers (IPL and OPL). OPL is composed of fibres and synaptic reticulum formed by interactions between photoreceptors and cells in INL. IPL in contrast is much wider and is comprised of GCL dendrites interlacing with INL neurons. In AD, increased tau phosphorylation has been observed in these layers in comparison to normal retinal tissues. pTau and the staining of tau paired helical filaments, in addition to microglial activation, RGC loss, and the impairment of retinal function, have been reported in 3xTg-AD and APP/PS1 animal models of AD (Chiasseu et al., 2017; Grimaldi et al., 2018; Gupta et al., 2016b) (Fig. 4). In addition, authors have also shown increased pTau immunoreactivity within the inner retinal layers of human AD postmortem tissue using different antibodies (den Haan et al., 2018c). This staining also co-labelled with RGC marker, TUJ 1 which is 50 kDa neuron specific β3 tubulin, further indicating that these cells are prominently affected in the disease process (Grimaldi et al., 2019) (Fig. 4).

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findings from human studies, total tau protein expression was not found to be significantly altered in the APP/PS1 mouse retina, even though significant increases in the levels of pTau (Ser404) were noted (Mirzaei et al., 2019b). Increased pTau in APP/PS1 animals has also been shown to correspond to levels of the protein in the CSF in 6-month-old mice (Chiasseu et al., 2017). Interestingly, while increased pTau reactivity has been reported around Aβ deposits in the brain (Ittner and Gotz, 2011), not much is known about their relative localization within the retina, however, increased levels of Aβ have been reported in the GCL while pTau reactivity in addition to the GCL has also been observed in IPL and OPL layers of the retina. The accumulation of Aβ and pTau aggregates in the retinal tissues of both human AD patients and mouse models has been reported by some labs (Grimaldi et al., 2018, 2019; Gupta et al., 2016b; Koronyo et al., 2017; Zhao et al., 2013). It is important to mention that there are also studies that have been unable to replicate neuropathological Aβ deposition in the retina (den Haan et al., 2018c; Ho et al., 2014; Schon et al., 2012; Williams et al., 2017). Therefore, caution is warranted against generalizations, as changes in Aβ and tau phosphorylation could also be caused by ageing or other retinal diseases that elicit overlapping molecular pathologies, including for example glaucoma (Gupta et al., 2008; Mirzaei et al., 2019b). Further studies involving different neurodegenerative diseases will enhance the accuracy and specificity of utilizing retinal changes in amyloid deposition and tau phosphorylation to detect AD.

2.3. Molecular imaging and the identification of AD markers in retina

In the last decade scientists have increasingly focused on investigating retinal changes in AD using various molecular imaging technologies. The reports of diffused protein aggregates and fibrillar deposits in the retina, suggests that the molecular signs of AD may overlap to a certain extent with the brain. In attempt to detect and monitor AD specific differences, studies have centered on both endogenous changes in the retina as well as the use of exogenous non-toxic staining agents, such as that can bind to Aβ. The morphology of these deposits in the retina, however, is different from plaques in the AD brain and this may hamper visualization in similar ways that are used to detect brain deposits.

2.3.1. Curcumin imaging of retinal AD changes

Curcumin, or diferuloylmethane, is a natural fluorescent polyphenol that is obtained from turmeric rhizome and exhibits an affinity for Aβ aggregates (Fig. 5). Curcumin is a food constituent and considered to be safe for administration by US FDA. Chronic oral administration of curcumin formulation Longvida was shown to have beneficial effects on mood and cognition in older population (Cox et al., 2015). Furthermore, its specificity has been validated by co-labelling with specific monoclonal antibodies against amyloid β peptide (den Haan et al., 2018c; Koronyo et al., 2017). The compound has also been shown to efficiently cross both the blood–brain and the BRB and can thus reach retina when delivered systemically, however >99% is directly conjugated by the liver, so the circulatung curcuminoids are either sulfates or glucuronides (den Haan et al., 2018b). Curcumin and its conjugates bind to β-pleated sheets of Aβ, as well as its oligomers, fibrils and plaques with high affinity, and also stains PHF-tau neurofibrillary tangles to some extent (Mutsuga et al., 2012). More recently, the fluorescence imaging of amyloid deposits using curcumin–Aβ interactions has been applied to image Aβ deposits in the retina. The optical imaging of curcumin fluorescence in the retina was shown to juxtapose with Aβ deposits in live retinal imaging of AD subjects as well as in post-mortem samples, thus

Fig. 5. Curcumin staining of the retinal amyloid (A) Schematic representation showing curcumin (ball and stick model) binding to Aβ (space filled model). Binding of curcumin to Aβ deposits leads to fluorescent signal from the retina. (B) Representative magnified images demonstrating retinal in vivo detection of curcumin spots in superior temporal region of human retina in healthy control and AD patient. Red circles highlight retinal spots attributed to curcumin-increased fluorescence. Scale bar: 400 μm. Reproduced with permission of American Society for Clinical Investigation for JCI Insight. 2017 2(16): e93621, https://doi.org/10.1172/jci.insight.93621, (Koronyo et al., 2017); conveyed through Copyright Clearance Center, Inc. (C) Curcumin staining of human AD post-mortem brain (hippocampus) and retinal tissue sections. There is an intense signal in fibrillary plaques which co-stains with 6E10 in the hippocampus. In retina, significant curcumin staining was not evident although there was intracellular APP reactivity against 6E10 antibody. Scale bar 50 μm. Reused from (den Haan et al., 2018c) under the Creative Commons Attribution 4.0 International License: Creativecommons.org/licenses/by/4.0/.
making it a promising tool for monitoring AD pathology (Koronyo-Hamauoi et al., 2011; Koronyo et al., 2017). Following curcumin administration, Koronyo et al. (2011) demonstrated that Aβ deposits were detectable in the 2.5-month-old APP/PS1 mice retina, which is much earlier than when Aβ plaques are reported to be formed in the brain. In this study, they correlated the observed fluorescence changes with progressive brain pathology in APP/PS1 mouse model (Koronyo-Hamauoi et al., 2011; Koronyo et al., 2017). Curcumin staining followed by optical imaging of the whole mounted retinas revealed a two-fold increase in the amyloid intensity in human AD post-mortem in comparison to that of control samples, and the staining of Aβ deposits was shown to correspond with the neuronal loss in the retina (Koronyo-Hamauoi et al., 2011; Koronyo et al., 2017). Subsequent studies revealed curcumin labeling within the retinas of living patients with amyloid depositions occurring mainly near the central and peripheral regions of the superior quadrant of the retina. The live retinal imaging of AD patients also exhibited a fluorescence pattern similar to that of the amyloid staining previously reported in human post-mortem retinas (Koronyo et al., 2017) (Fig. 5).

Axonal loss and NFL thinning in AD patients has been acknowledged previously, and mainly occurs in the superior regions of the retina that also demonstrate increase in amyloid pathology. Neuronal loss in the superior quadrant and the mid- and far-peripheral regions of the retina was initially observed by Blanks et al. (1996) (Blanks et al., 1996b). More recent investigations have demonstrated mRGC loss and Aβ accumulation in the same superior quadrant region, supporting the prior work (La Morgia et al., 2016). Another study has reported that upon curcumin administration, patchy hypo-fluorescent signals were identifiable using fundus autofluorescence and OCT in the areas surrounding macula and vascular regions of the retinas of MCI patients (Kayabasi et al., 2014). Another group however has shown that while curcumin and 6E10 antibody co-stained amyloid deposits in the hippocampus, only 6E10 antibody positive APP/Aβ staining was evident in the extracellular space in human postmortem AD retinal tissues (Fig. 3) (den Haan et al., 2018c). Studies at Alzheimer’s Centre Amsterdam also indicate that amyloid deposition signal in the retina was not detected upon oral administration of Theracurmin, Novosol and Lonviqua curcumin formulations (unpublished data). The paucity of literature on this topic and conflicting observations emphasize that additional studies in different cohorts may be required to establish the sensitivity and specificity of retinal curcumin staining to achieve clinical utility in AD patients. In addition to the retinal imaging modality, infrared laser confocal quasi-elastic light scattering procedure and the fluorescent ligand eye scanning techniques (Kerbage et al., 2015) have ascertained amyloid pathology in the lens using extrinsic fluorescent dyes. However, cataracts and corneal pathologies can significantly confound lens imaging analyses for AD in elderly cohorts.

### 2.3.2. Hyperspectral imaging of retinal AD changes

More recently, there have been attempts to distinguish Aβ deposition in the retina using hyperspectral scanning (HSI) (Hadoux et al., 2019; More and Vince, 2015; Sharafi et al., 2019). HSI does not require extrinsic fluorophore labelling and works by acquiring and analysing adjacent wavelength spectra within a given range. This technique takes advantage of the light scattering properties of small particles in tissue, which is governed by Rayleigh scatter principle. Optical and digital processing of the sequential HS images allows non-invasive discriminating structural and biochemical changes in the tissues. In the retina, small soluble Aβ oligomers are believed to contribute to an HSI signature most salient in the short visible wavelength spectrum. The proof-of-principle studies demonstrated that HSI spectral signatures from AD post-mortem brain tissue differed significantly in comparison to that acquired from normal age-matched individuals. Similar patterns of the spectral variation in wavelengths have also been observed between normal and Aβ1–42 peptide treated SHSY5Y cells (More and Vince, 2015). Interestingly, an analysis of retinal tissue samples from normal and AD subjects offered similar differentiating results. Total wavelength scattering in samples from AD patients was diminished, suggesting its dispersal by protein aggregation neuropathology, or associated structural alterations. Ex vivo HSI imaging of retinal tissues from a mouse model at different stages of the disease, supported these observations, with significant differences observed at 4 months, which increased exponentially at 6 months of age. At 8 months, APP/PS1 mice have some Aβ plaque formation, and accordingly, discernible differences in HSI were further observed between the control and transgenic mice at this stage (More and Vince, 2015). Intriguingly, at 4 months of age, amyloid deposition is not evident in APP/PS1 mouse brains, which suggests that the light scattering properties of small soluble Aβ species preserves the HSI signature even in the absence of insoluble plaques, and this property makes this approach a potentially useful tool for early detection of AD (Fig. 6).

The translation of HSI technology to human in vivo imaging has proven to be challenging with significant inter-subject spectral variability. Hadoux et al. (2019) recently demonstrated that HS can discriminate between individuals who have high Aβ load from those with less Aβ load on cerebral PET scanning (Fig. 6). However, owing to a considerable inter- and intra-subject ocular reflectance variability within different regions of the retina, HSI data in its unprocessed form was not remarkably distinguishable between AD and control subjects. Following the normalization of the spectral features with known spectral interference caused by various proteins and pigments in the ocular tissues, spectral discrimination was observed between AD and control subjects, and results largely corresponded with the spectral profile of Aβ in solution (Hadoux et al., 2019). The processed data was able to make a distinction between retinal milieu of AD and control subjects. Again, there was a significant degree of variation within the retina depending on which region was examined, and HS scores were particularly high and discriminatory in the superior and foveal regions between AD and control subjects. Although, the exact distribution of various Aβ species and their production in the retina remains poorly defined, higher levels of Aβ plaque-like formations have been reported in the superior quadrant previously (Koronyo et al., 2017). While the discriminatory signature of retinal HSI from human AD subjects and AD mice models likely arises from the accumulation of Aβ, there are many other factors that complicate the scenario and could influence spectral shift such as metal ion accumulation, changes in pTau, inflammatory processes, and various other structural and biochemical changes could play a role (Chiasseu et al., 2017; Gupta et al., 2005). Although it is also possible that changes in vascular tortuosity and anterior segment tissues affect the spectral measurements of the retina; wavelengths in the range of 700–900 nm also exhibited significant differences (Sharafi et al., 2019). Due to the increased tissue penetration afforded by the longer wavelength of near infrared light, it is less likely to have been significantly affected by ocular tissues in the anterior segment. Broadly, this emerging imaging application is not without drawbacks, as a combination of intertwined biochemical changes and structural variations could alter the hyperspectral signal. These findings warrant further investigation for a clear understanding of the mechanisms and their validation in different cohorts. A few other studies have also noted localized fundus auto-fluorescence signals using SD-OCT from at-risk AD subjects, that positively correlated with amyloid β florbetapir-PET imaging scores, further supporting the hypothesis that the retinal changes in AD might be associated with brain pathology (Snyder et al., 2019). The chronic effects of Aβ accumulation on ganglion cell death in the retina and impact of cellular loss on intrinsic or exogenous fluorophore mediated imaging are not known. The imaging of ganglion cell death in real time in glaucoma patients, as well as in glaucoma and AD animal models, has been recently demonstrated (see below) and some effects of these degenerative changes could contribute to the aforementioned differences (Cordeiro et al., 2017). Notwithstanding, ongoing optical, and analytical software developments in HSI camera technology will refine its sensitivity and specificity to detect AD changes. This approach
has the potential to screen individuals in combination with other biomarkers of disease to increase the predictive power. It could also likely find applications in studying intrinsic wavelength signature differences in other ocular disorders, such as glaucoma and AMD.

### 2.3.3. Imaging of cellular apoptotic changes

Apoptotic activation and cell death constitute the key pathological drivers for neuro-degenerative processes in AD. Hence, cell death markers have been the subject of intense research and development to track apoptotic cell imaging within the retina. Novel techniques to identify cells undergoing apoptosis in the retina has the potential to not only help to diagnose the disease much earlier than in the brain, but can also provide valuable insights to the molecular mechanisms by which apoptosis may affect neuronal loss in AD, and how these stages may be targeted by future therapeutic agents. For example, externalisation of membrane phospholipid phosphatidylserine (PS) in apoptotic cells has emerged as a promising early marker for cell death. PS is able to bind with Bis(zinc(II)-dipicolylamine) (Zn-DPA) compound (Kwong et al., 2014). NMDA induced toxicity studies have shown that intravitreal administration of Zn-PDA- fluorescein conjugates successfully detect apoptotic cells during live retinal imaging (Kwong et al., 2014). This technique can also identify RGC apoptosis in rodent models much earlier than changes in DNA fragmentation (Kwong et al., 2014; Mazzoni et al., 2019). Recently, Zn-PDA conjugated with a Texas-red fluochrome (PSVue-550) was able to detect apoptotic photoreceptor cells when instilled via eyedrops (Mazzoni et al., 2019). Retinal electrophysiological studies further revealed no significant functional changes in the administered eyes, indicating that the conjugate was pharmacologically safe whilst eliminating the potential side-effects caused by repeat intravitreal injections (Mazzoni et al., 2019). Similar to Zn-PDA, the radio- labelled annexin-5 protein when administered intravenously, efficiently labelled the RGCs undergoing apoptosis both in glaucoma patients and in animal models of glaucoma and AD (Cordeiro et al., 2010, 2017). Fluorescently labelled annexin-5 interacted with apoptotic RGCs in various injury models and was effectively tracked by real-time imaging with scanning laser ophthalmoscopy (Cordeiro et al., 2004). This approach was also exemplified in a triple transgenic model of AD, whereby retinal apoptosis was observed in its various phases ranging from early to late apoptosis and necrosis (Cordeiro et al., 2010). This detection of apoptosing retinal cells (DARC) using annexin-5 has since been translated to human studies in phase I clinical trial and initial proof-of-concept studies demonstrate that intravenous annexin-5 labelled with fluorescent dye (ANX776) is pharmacologically safe and well tolerated in glaucoma patients (Cordeiro et al., 2017). Similar approaches may also be useful in monitoring early cellular degenerative changes in the retina in AD patients. Akin to annexin-5, propidium iodide (PI) dye can also differentiate apoptotic and necrotic neuronal cell populations in an animal model of focal ischemic stroke (Bahrami et al., 2011). These in vivo imaging advancements to track changes in early cell death markers suggest that cellular degenerative changes in AD can potentially be detected and monitored in the retina much more effectively than is possible in the
3. Neurodegenerative changes in the retina in AD

3.1. Retinal structural changes in AD patients

Since initial reports that highlighted the loss of RGCs, and particularly melanopsin expressing RGCs (mRGCs) in AD (Sadun and Bassi, 1990; Blanks et al., 1989), several studies have published findings that support the degenerative impact of AD on the inner retinal arbor architecture, including the loss of RGCs in the foveal and para-foveal regions (Blanks et al., 1996b). These findings were supported by post-mortem studies, which revealed a loss of approximately one-third of all neurons in the retina, particularly distributed in the superoinferior region, with a loss of approximately one half in the mid-peripheral region, and the greatest losses within the inferior peripheral regions of the retina (Blanks et al., 1996a). Since the initial structural changes in the retina reported in AD subjects using cross-sectional OCT imaging studies (Paris et al., 2001), there have been tremendous improvements in OCT technologies, which now provide better resolution and analysis algorithms for layer segmentation in vivo. Significant thinning of both the naso-temporal and superoinferior regions of the RNFL in AD subjects, in addition to the thinning of the inner and outer macular rings was observed (Ascaso et al., 2014). Other reports indicate retinal changes that are predominantly focused on the superior and inferior regions of the RNFL (Doust et al., 2017; Lu et al., 2010). Another report has indicated the loss of the GCL and IPL, the preferential thinning of the superior and inferior quadrants of the RNFL, as well as the thinning of the peripapillary region of the RNFL, where RGC axons converge in early AD subjects (Lopez-de-Eguileta et al., 2019). This study suggested that RNFL, IPL and GCL changes might be useful for AD diagnosis, however Bruch’s membrane opening–minimum rim width (BMO-MRW) remained unchanged. Thus, advancements in ocular imaging have provided evidence of inner retinal thinning and loss of the retinal nerve fiber layer (RNFL) in AD and its preclinical and prodromal stages, although some studies have also suggested a non-significant relationship between the effects of AD and changes in retinal imaging by OCT (den Haan et al., 2019; Haan et al., 2019a; Sanchez et al., 2020; Sanchez et al., 2018). An initial meta-analysis concluded that there was a significant RNFL thinning in early stages of AD (Knoll et al., 2016). Another meta-analysis of 887 AD, 216 MCI and 864 control subjects from 25 different studies revealed reduced macular thickness in AD subjects and peripapillary RNFL thinning in AD and MCI subjects compared to the controls (den Haan et al., 2017). More recently, a further meta-analysis of 30 cross sectional studies that included 1257 AD, 305 MCI and 1460 control cases, supported these observations and indicated peripapillary RNFL loss and choroidal thinning, in addition to macular changes such as GC-IPL and ganglion cell complex (GCC) deficits and loss of macular volume and thickness, in MCI and AD subjects (Chan et al., 2019). Overall, these studies indicate that retinal monitoring to detect changes in RNFL and macula GCC may be an important additional measure to assess disease progression when combined with other assessments; but the differences are expected to be small, and may not be directly useful in clinical practice.

Retinal changes in patients with AD and MCI have been demonstrated to correlate with neuropsychological changes. Significant loss of the thickness of the RNFL and GCL-IPL complex was observed in subjects with MCI and AD, which was found to correlate with mental state examination (MMSE) scores (Ferrari et al., 2017). MMSE is a commonly used clinical test to assess cognitive function. MMSE defects implicate that changes in retinal and brain pathologies could show congruence to some extent (Liu et al., 2015). Other studies have corroborated this and shown that changes in MMSE were significantly correlated with the losses in superoinferior regions of the RNFL and average peripapillary RNFL thickness (Cunha et al., 2017a). These changes in cognitive parameters were concomitant with macular changes, such as changes in average macular thickness and GCL-IPL loss (Cunha et al., 2016b, 2017a). Other studies have added to the growing body of evidence and reported similar correlations between changes in MMSE and values for RNFL thickness in various regions of the retina in AD and MCI patients (Oktem et al., 2015; Shen et al., 2014). For instance, a potential association between certain episodic memory changes and RNFL thickness in MCI was observed, particularly in the inferior quadrant region of the retina (Shen et al., 2014). The involvement of the macular region was further supported by findings that MMSE scores, which reflect cognitive impairment in AD subjects, correlated with the loss of macular volume (Iseri et al., 2006). In agreement with previous cross-sectional reports, the correlation between neuropsychological changes and defects detected via retinal imaging was established in a one-year longitudinal study. This study provided evidence that shifts in peripapillary RNFL thickness were concomitant with progressive changes in clinical dementia rating (CDR) and AD assessment scale-cognitive subscale (ADAS-Cog) tests (Trebbastoni et al., 2016). A UK Biobank, three year longitudinal cohort study also found that changes in RNFL thickness in OCT scans were associated with cognitive decline as measured with prospective memory, pairs matching, reaction time and reasoning based analysis (Ko et al., 2018). Thus, there is evidence for a correlation between specific inner retinal changes and cognitive decline in cases of AD, supporting the theory that retinal changes may be related to CNS pathologies. Our research team also observed a significant difference in retinal GCL thickness in a cross-sectional study of control, pre-clinical AD, and clinical AD groups (Golzan et al., 2017). The association between changes in retinal GCL thickness and neocortical Ap standardized uptake value ratio (SUVR) in PET scans in preclinical AD subjects was found to be not significant. Similarly, the correlation between RNFL thickness and neocortical Ap standardized uptake value ratio in the control and preclinical cohort was not significant. There was, however, some decrease in RGCL thickness corresponding to the macular region in clinical AD cases in comparison to the control and preclinical AD subjects (Fig. 7).

As mentioned above, although spectral domain OCT-based techniques are promising, they lack specificity for AD, as the thickness of various retinal layers is highly variable in the normal population and is also affected by other age-related neurodegenerative disorders of the eye and the brain such as age related macular degeneration (AMD), glaucoma (Gupta et al., 2016a), demyelinating optic neuritis, multiple sclerosis (MS), and Parkinson’s disease (PD) (Moreno-Ramos et al., 2013; You et al., 2018). Patients with frontotemporal dementia also have been shown to exhibit a significant reduction in peripapillary RNFL thickness, similar to that reported in AD patients (Ferrari et al., 2017). Retinal parameters could also be secondarily altered in vascular disorders such as diabetes or hypertension (Cheung et al., 2017; Ng et al., 2016). Therefore, the detection of additional effect of AD is likely to be complicated by several factors, particularly in cross-sectional studies. Longitudinal measurements hold the promise of measuring the effects of diseases while minimizing statistical variations, but concurrent pathology may complicate interpretation. Investigations into the distinct effects of AD on specific regions of the retina is likely to enhance the clinical value of OCT in disease diagnosis.

3.2. Functional changes in AD patient retinas

In addition to the documented laminar structural changes in various studies, retinal functional deficits have also been reported in AD patients. Significant delays in latency, which indicate a disruption in signal processing and decreased amplitude, were observed in various components of pattern electroretinogram (pERG) and were correlated with overall changes in the thickness of the RNFL in AD patients (Katz et al.,...
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However, the caveat is that the visual field periphery constriction has been linked to the severity of AD. These differences in pERG and VEP support the premise that AD performance may also become impaired in more severe AD cases due to lack of concentration and not just sensory loss.

In contrast, other studies argue that color vision in AD patients. While information about other functional changes that can be detected via electrophysiological measurements is limited, it can offer useful insights into the neuro-retinal effects of AD.

Retinal structural and functional changes in animal models of AD

Animal models of AD illustrate deficits in inner retinal thickness and electrophysiology. Our studies have revealed that APP/PS1 mice exhibit increased levels of soluble and aggregated forms of Aβ in their retinas. APP/PS1 is a mouse model of AD that expresses human APP transgene with Swedish mutation, which involves a double mutation involving Lys670Met and Asn671Leu sites along with Leu166Pro mutation in PSEN1 gene. The expression of both these transgenes is regulated under the control of Thy1 promoter (Radde et al., 2006). Recent studies have demonstrated aggregation of Aβ in the endoplasmic reticulum and its neurotoxic effects (Meli et al., 2014), as well as the formation of neuritic plaques in the extracellular space from degenerating neurons. A diminution of the neuronal density in the GCL, as well as a significant thinning of the IPL, was observed in 13-16-month-old APP/PS1 in our study, indicating that the inner retina was preferentially affected in these animals. Increased TUNEL staining—which is an indication of cells undergoing apoptosis—was also observed in the inner retinal layers (Gupta et al., 2016b). An increase in the expression of the microglial/innflammatory F4/80 cell surface glycoprotein antigen and monocyte chemoattractant protein-1 in the GCL of APP/PS1 animals has also been reported in other studies (Gupta et al., 2016b; Perez et al., 2009). Consistent with this, optic nerve sections have been shown to exhibit a reduction in axonal density in APP/PS1 mice in Bielschowsky’s silver staining, while a reduction in retinal thickness has previously been described in a Tg2576 AD mouse model (Liu et al., 2009). Interestingly, there are growing reports of increased IPL thickness in the early stages of AD, and one mechanism to explain this paradoxical inflation is the increased neuroinflammation in...
the synaptic layers of the retina (Liu et al., 2019; Snyder et al., 2016). These changes however are reduced with disease progression and completely reversed in late stage AD where a thinning is observed. This is in agreement with reports that nearly one-third of the Aβ plaques are invariably identified in the INL, resulting in a significant decrease in IPL thickness in end-stage AD models (Fig. 8C) (Perez et al., 2009). Aβ deposition in the GCL could further explicate ganglion cell degeneration and the abnormal ERG patterns reported in rodent models of AD (Gupta et al., 2016b). The INL is principally composed of cell bodies involved in the vertical transmission of information from photoreceptors via their dendrites and axonal connections. Hence the accumulation of Aβ in these segments can disrupt their normal function, impacting on synaptic transmission and ultimately lead to a decrease in the amplitudes of inner retinal electrophysiological responses observed in mice models of AD. Postsynaptic density protein 95 (PSD-95) which is located in the postsynaptic axonal regions, showed reduced density in AD and epigenetic editing of the gene rescued cognitive deficits in APP/PS1 mice (Bustos et al., 2017). The protein was also shown to be reduced in RGCs in microbead induced glaucoma models indicating synaptic loss (Williams et al., 2016). In ageing APP/PS1 mice, the degenerative changes in the RGC dendritic arbour were observed to precede cell loss (Williams et al., 2013). The accelerated RGC dendritic atrophy and pruning is likely attributed to the deposition of Aβ and other protein aggregates in proximity to synaptic junctions and axons. As a consequence of retinal Aβ deposition, amplitudes of positive scotopic threshold response (pSTR), which is an important element of the dark-adapted ERG were observed to be reduced in APP/PS1 mice. pSTR peak arises at approximately 120 millisecond after very dim light stimulus and primarily reflects inner retinal function in rodents (Bui and Fortune, 2004; Gupta et al., 2014b, 2016b). STR is considered to be a predominantly RGC-driven component and deficits have previously been reported in human glaucoma as well as in primate models of optic nerve injury (Frischman et al., 1996; Korth et al., 1994). APP/PS1 mice between the ages of 13–16 months were shown to exhibit significant pSTR differences with amplitudes reduced to approximately half in comparison to wild type mice (Fig. 8D). Additional evidence of electrophysiological deficiencies in AD pathology comes from animal models, with abnormal flash VEP measurements reported in APP/PS1 mice (Gao et al., 2015a).

Observed reductions in a-waves which represent rod cell function, and b-wave amplitudes which primarily represent bipolar cells and other interneurons have been reported in other studies and are suggested to be affected by Aβ accumulation (Ning et al., 2008). Interestingly, we detected that APP/PS1 animals have slightly elevated intraocular pressure (IOP) 12.1 ± 0.86 mmHg in APP/PS1 mice vs 9.8 ± 0.67 in WT animals. This could potentially be elicited by nucleation of Aβ aggregates and their deposition in the trabecular meshwork and interconnecting blood vessels, which can impede the outflow facility. However, the exact underlying cause of IOP elevation, or its impact on retinal changes in these animals, remains unclear (Gupta et al., 2016b).

4. Retinal vascular changes in AD

Epidemiological information has suggested a role for vascular involvement in the pathophysiology of AD (Bell and Zlokovic, 2009). Various examples of vascular pathology have been described at post-mortem in approximately 75% of all AD cases (van der Flier et al., 2018); while approximately 85–95% of patients demonstrate substantial Aβ deposition in blood vessels, as well as demonstrate other vascular abnormalities in the brain (Viswanathan and Greenberg, 2011). These vascular changes include chronic ischemia of various sized vessels, lacunar infarcts, atherosclerosis, and widespread cerebral amyloid angiopathy associated with microhemorrhages (Charidimou et al., 2017). A broad array of research has established an association between the cardiovascular parameters of arterial blood pressure and markers of arterial stiffness including, pulse wave velocity and pulse pressure, arterial stiffness, reduced endothelial cell response to stress, carotid intimal-medial width, on the one hand, and declining cognitive function on the other (Hajjar et al., 2016; Zeki Al Hazzouri et al., 2013). Thus, there is a considerable interaction between AD and cerebrovascular disease and due to overlapping symptomatology it may be at times, difficult to clinically differentiate AD from mixed dementias. The differential effects of mixed dementias on the retina are not known, however, it is acknowledged that concomitant pathology may have additive or synergistic ocular effects and make the interpretation challenging.
Once a clear validation of the scope of vascular changes in AD is established, it can be incorporated as a potential diagnostic measure to the NIA-AA research framework and scheme could likely be updated as ATV(N) (Jack et al., 2018).

Multiple groups have actively pursued the potential correlation of AD-associated changes in the brain with various aspects of static and dynamic vascular parameters. Emerging evidence alludes that parallel vascular alterations in the retina could be indicative of the initial neurovascular dysfunction observed in AD. A wide range of abnormalities—including constricted retinal vessels with increased tortuosity, microinfarct development, atherosclerosis, amyloid angiopathy, endothelial cell dysfunction, reduced arterial fractal dimensions, and changes in branching patterns of vessels—have been reported in AD (Golzan et al., 2017; Lesage et al., 2009). Sharafi et al. (2019) recently showed that subjects with higher levels of Aβ in the brain (Aβ+) also exhibit considerably more retinal vein tortuosity in comparison to people with lower Aβ scores in PET (Aβ-). In addition, reductions in blood flow and increased blood oxygen saturation levels, akin to those reported in the brains of patients with AD pathology, have also been documented in the retina (Peke et al., 2015; Olahsdottir et al., 2018). The Rotterdam study initially launched in 1990s, identified a correlation between retinal vessel caliber and cognitive diminution, with data indicating that retinal venular widening is associated with an increased risk of vascular dementia (de Jong et al., 2011). Imaging studies involving the retinal fundus have also described increases in venous diameter and decreases in arterial diameter in conjunction with reduced fractal dimensions in AD subjects (McGrory et al., 2017). However, the arteriolar diameter of amyloid-positive subjects by PET was found to be higher than the amyloid-negative subjects in a zone adjacent to the optic nerve head (Sharafi et al., 2019). Another recent study that outlined the tightening of fractal dimensions of retinal arterioles in cases of vascular cognitive impairment did not identify differences between control and AD groups (Jung et al., 2019). Several studies have also described choroidal attenuation in cases of dementia via OCT enhanced depth scanning (Cunha et al., 2017b; Wong et al., 2011), thereby further corroborating the evidence of a relationship of vascular attenuation in AD. The visual disturbances in AD could also be attributed to the local atrophy of retinal and choroidal vessels or endothelial disintegration arbitrated by Aβ toxicity (Berisha et al., 2007).

In addition to the extracellular deposition, amyloid plaques and aggregates are extensively distributed proximately to the blood vessel walls and networks in the AD brain (Bu et al., 2018). This is similar to that reported in cerebral vascular amyloid pathology (Koronyo et al., 2017). Owing to endothelial and physiological similarities in the micro-vessels of the retina within the brain, amyloid deposition could restrict the flow of blood in smaller vessels in the retinas of AD patients. Murine models of AD likewise manifest augmented vascular rigidity in response to amyloid build up in blood vessel walls, which impede blood flow and induce leaks owing to compromised permeability (Tanifum et al., 2014). Aβ deposition was also found to be associated with damage to capillaries in 14-month-old Tg2576 mice retinas (Dutescu et al., 2009). Endothelial cell dysfunction is an additional factor that could influence APP metabolism in these cells in culture and potentially in branching patterns of vessels—have been reported in AD (Golzan et al., 2017; Lesage et al., 2009). Sharafi et al. (2019) recently showed that subjects with higher levels of Aβ in the brain (Aβ+) also exhibit considerably more retinal vein tortuosity in comparison to people with lower Aβ scores in PET (Aβ-). In addition, reductions in blood flow and increased blood oxygen saturation levels, akin to those reported in the brains of patients with AD pathology, have also been documented in the retina (Peke et al., 2015; Olahsdottir et al., 2018). The Rotterdam study initially launched in 1990s, identified a correlation between retinal vessel caliber and cognitive diminution, with data indicating that retinal venular widening is associated with an increased risk of vascular dementia (de Jong et al., 2011). Imaging studies involving the retinal fundus have also described increases in venous diameter and decreases in arterial diameter in conjunction with reduced fractal dimensions in AD subjects (McGrory et al., 2017). However, the arteriolar diameter of amyloid-positive subjects by PET was found to be higher than the amyloid-negative subjects in a zone adjacent to the optic nerve head (Sharafi et al., 2019). Another recent study that outlined the tightening of fractal dimensions of retinal arterioles in cases of vascular cognitive impairment did not identify differences between control and AD groups (Jung et al., 2019). Several studies have also described choroidal attenuation in cases of dementia via OCT enhanced depth scanning (Cunha et al., 2017b; Wong et al., 2011), thereby further corroborating the evidence of a relationship of vascular attenuation in AD. The visual disturbances in AD could also be attributed to the local atrophy of retinal and choroidal vessels or endothelial disintegration arbitrated by Aβ toxicity (Berisha et al., 2007).

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White matter hyperintensity changes have also been shown to be correlated with retinal fractal dimensions in AD retinas (Jung et al., 2019). These changes in various retinal vascular measures may be reflective of different stages in AD pathology. Furthermore, some studies have described decreases in the central retinal artery and vein equivalent (CRVE/CRAE) and the fractal dimension of the arteriolar and venular networks, as well as altered curvature tortuosity of the arterioles and venules, in AD patients (Cheung et al., 2014; Williams et al., 2015). OCT-angiography (OCTA) advances have made it possible for ophthalmologists to obtain insights into capillary bed changes in the retina in a clinical setting. While some studies have reported a decline in vessel density and blood flow and an increase in the foveal avascular zone (FAZ) area in early AD using OCTA imaging, studies carried out by other groups have failed to detect any differences in these measures (Bulut et al., 2018; Haan et al., 2019b; O’Bryhim et al., 2018). A reduced vascular and perfusion density in the macular regions was reported recently using OCTA in AD compared to MCI and control cases (Yoon et al., 2019). More recently, significantly higher vascular density around macular and optic nerve head regions was observed, with no significant FAZ area differences in preclinical stages of AD, as determined by Aβ+ PET scans (van de Kreeke et al., 2020).

In addition to static vascular changes, the association of dynamic measures such as vessel pulsation has been examined in AD. Spontaneous retinal venous pulsations is a normal finding in humans and is believed to originate from interaction between IOP, CSF pressure, ocular perfusion and central retinal vein pressures (Golzan et al., 2017). Our investigations into the association between retinal hemodynamics and cerebral Aβ load in preclinical AD subjects revealed a negative correlation between the amplitude of retinal venous pulsations and Aβ levels, indicated as SUVr in PET scanning. Of note, a positive correlation was detected between retinal arterial pulsation amplitudes and Aβ SUVr in pre-clinical AD (Golzan et al., 2017). We also observed a similar trend in clinically diagnosed AD subjects, which supports an association between retinal hemodynamics and AD (Fig. 9). Reduced dilation of arteries in the retina of AD patients in response to light flicker by dynamic vessel analyzer (DVA) further attests to ongoing changes in blood-flow parameters and neurovascular decoupling (Querques et al., 2019). Although we are uncertain of the significance of these findings, chronic vascular changes and/or sustained high arterial pulse amplitudes could contribute to degenerative changes in the brain as well as inner retina and macular volume deficits.

In conclusion, identifying and characterizing new biomarkers for vascular changes in the retina will provide mechanistic insights into the disease process and could complement current diagnostic tools for AD (Golzan et al., 2017). Currently, our knowledge of the vascular changes in AD including alterations in perivascular spaces, microbleeds and small infarcts mainly arises from brain MRI. It is to be emphasized that various aforementioned vascular parameters could be affected by age and other ophthalmological comorbidities such as glaucoma, diabetic and hypertensive retinopathy and a further limitation is that not all studies used amyloid proven AD cases. In addition, individual variations in the retinal layers, vascular thickness, spherical equivalent and image
quality could contribute to these differences.

5. Other biomarkers of AD in the retina

5.1. Retinal proteomics changes in AD

While the reports of retinal changes have been increasing in AD, there is inadequate information regarding the molecular pathways that are impacted at different stages of the disease. A greater understanding of these progressive molecular changes will untangle the mechanistic basis of the functional, imaging, and histological changes observed in the retina in AD. Knowledge of region specific proteomics changes will enhance our ability to explain localized neural/vascular and imaging deficits in the retina. While proteomic changes in the blood and CSF have been investigated in detail, and more information has started to emerge from brain tissues of human AD post-mortem and transgenic animal models (Bai et al., 2020; Johnson et al., 2020); AD retinal tissues remain relatively under-investigated.

5.1.1. Proteomics changes in the retina of APP/PS1 mouse model

Our retinal proteomics investigations in APP/PS1 transgenic mouse model of AD demonstrated significant protein expression changes at 2.5 months, much before Aβ aggregation pathology is evident in the brain of these animals (Mirzaei et al., 2019b). These changes were compared with findings in 8-month-old APP/PS1 mice retinas. At this age, APP/PS1 mice exhibit cognitive, learning, and behavioral deficits, but without the neuronal cell loss that accompanies later stages of the disease in this model, which can confound protein expression analysis due to widespread senescence and tissue atrophy (Gengler et al., 2010; Radde et al., 2006).

Proteomics analyses have revealed insights into the activation of various biochemical processes, such as phagosome maturation, ubiquitination, mitochondrial function, amyloid processing, oxidative stress, and the ER stress response (Mirzaei et al., 2019b). There were approximately 2000 proteins identified, of which 50 and 36 proteins were altered at 2.5 months and 85 and 79 in 8-month-old mice retinas, with increased and decreased abundance respectively (Fig. 10). In 8-month-old retinas, the proteins involved in APP processing, proteasomal activation, and ribosomal networks were differentially regulated, reflecting...
the biochemical response to developing AD pathology (Fig. 11). APP, antioxidant enzymes peroxiredoxin 1, ribosome biogenesis protein Sbds, and ATPase enzyme Atp6v1e1 were enriched while the acetyl-galactosaminyl transferase Galnt13, transcription factor Crx, and mitosis regulator Mad2l1 were downregulated in the APP/PS1 retinas irrespective of the age. Intriguingly, levels of APP in the retina were elevated 2–3 fold in both young and older mice, providing evidence of retinal amyloid accumulation in this model much before the disease is manifested clinically. Furthermore, calyxntenin-1 and AP1S2 adapter proteins, that play roles in protein sorting within the Golgi network and regulating sensory axonal arborization respectively, exhibited a two-fold increase in young 2.5-month-old retinal tissues. This is in contrast to the expression of cleavage stimulation factor CSTF2, which is involved in polyadenylation signal and mediating 3′ cleavage of pre-mRNAs, which was increased two-fold in older APP/PS1 mice retinas (Mirzaei et al., 2019b).

Amongst the downregulated proteins, general transcription factor GTF3C2 and transcription regulatory protein cystatin B were most downregulated proteins in young mice retinas while crystallins BA1 and BB2 exhibited approximately five-fold downregulation in older mice tissues. With advancing pathology, APP/PS1 mice retinas depicted an increase in amyloid processing proteolytic enzymes such as cathepsin D, presenilin 2, and nicastrin. Our work also identified that proteasomal proteins Psma5, Psmd3 and Psm2 were remarkably enriched in the retina. This was supported by findings of significant downregulation of protein synthesis and elongation factor proteins such as Eef1a1, Rpl35a, Mrpl2, and Eef1e1 in older mice retinas. Overall, amyloid processing, cytoskeletal and microtubule organization, neuronal development and differentiation, apoptosis, and biological functions associated with neuritogenesis were the pathways most prominently affected (Mirzaei et al., 2019b). These differential changes in protein expression are characteristic of the progressive effects of AD on the retina with respect to age. While the significance of some of these molecular changes in AD retina may not be clear at this stage, proteomics data will serve as

Fig. 10. Differential protein expression changes in APP/PS1 mouse retina (A) List of 10 most upregulated and downregulated proteins in the 2.5 (left) and 8 month (right) old APP/PS1 mice retinas, respectively. (B) Overlap of the differentially regulated proteins between the 2.5 and 8-month-old APP/PS1 mice retinas (p value ≤ 0.05). The common differentially affected proteins in 2.5- and 8-month cohorts are listed. Reused with permission of Springer Nature for Mol Neurobiology, 2019, 56, pages 6017–6034 (Mirzaei et al., 2019b) (red, up and green, down-regulated).
important reference material for future studies. Similar studies need to be carried out in human AD postmortem retinas and knowledge generated will elucidate disease mechanisms and identify novel disease biomarkers. Analyzing proteomics data in conjunction with immunohistochemical analyses will establish the localization of specific markers to individual layers or cell types within the retina. Ability to isolate specific regions of the retina with the help of laser dissection microscopy or serial tangential section techniques is likely to ascertain the extent and significance of proteome alterations in various retinal cell populations (Kanan et al., 2010). Comparing retinal changes with specific regions of brain will demarcate the relative biochemical effects of AD on these two tissues and help to establish whether/or not the retinal tissue is affected much earlier in the disease process. Furthermore, comparing the effects of AD on the retina with other age-related retinal disorders such as glaucoma and AMD will clarify the specific effects of AD and molecular commonalities and differences between these neurodegenerative disorders (Mirzaei et al., 2017; Rozing et al., 2020).

5.1.2. Effects of Aβ on 661W retinal neuronal cell proteomics

The specific effects of Aβ42 peptide on 661W retinal neuronal cells has been studied by carrying out comprehensive time- and dose-dependent proteomic analyses, to understand the mechanisms underlying Aβ neurotoxicity (Deng et al., 2019). 661W is a mouse retinal neural precursor cell line that is shown to express opsin 1 cone-specific marker Opn1mw. The cells are also reported to express some of the RGC and neuronal cell markers such as Thy1, Brn3b, Tuj1, NeuN along with intermediate filament protein nestin, which plays a role in radial axonal growth (Sayyad et al., 2017). Similar to the changes observed in animal retinas, treatment with Aβ depicted time- and concentration-dependent perturbations of cellular pathways that are involved in protein synthesis, RNA processing, autophagy, cytoskeleton reorganization and oxidative phosphorylation (Deng et al., 2019). Overall, 5837 proteins were identified, out of which 380 proteins were differentially affected in response to treatments with various Aβ concentrations. Nuclear protein URB1 and U6 snRNA-associated protein LSm1, which plays a role in mRNA processing, as well as RNA binding protein 47 and nuclear ribonucleoprotein RBMX were enriched in response to treatment with Aβ. In contrast, levels of 60s ribosomal proteins such as Rpl29 and Rpl36a and 40s ribosomal protein RPS19 were reduced. Comparatively, cell cycle regulatory protein DPY30, which is involved in epigenetic transcriptional activation, and histone H3 acetylation were decreased in retinal neuronal cells subjected to treatment with Aβ for 24h (Deng et al., 2019). The canonical pathway analysis revealed that elf2, elf4, and p70S6K proteins which are involved in regulating transcriptional signaling pathways, were also differentially affected. Amyloid accumulation has previously been shown to associate with mitochondrial dysfunction in primary cortical neurons both in culture and in AD tissues (Cha et al., 2012). Aβ treatment indeed resulted in the downregulation of regulatory proteins involved in mitochondrial function and oxidative phosphorylation in retinal neuronal cells. Apart from these changes, authors also identified that proteins associated with autophagy, actin cytoskeleton, keratin filaments, spliceosome, and the lysosome were enriched, implicating these key cellular pathways to be affected by Aβ in these cells (Deng et al., 2019). Sirtuin signaling which is inversely associated with Tau accumulation in AD, was differentially affected in retinal neuronal cells subjected to treatment with Aβ (Yin et al., 2018). Sirtuins indeed resulted in the downregulation of regulatory proteins involved in mitochondrial function and oxidative phosphorylation in retinal neuronal cells. Apart from these changes, authors also identified that proteins associated with autophagy, actin cytoskeleton, keratin filaments, spliceosome, and the lysosome were enriched, implicating these key cellular pathways to be affected by Aβ in these cells (Deng et al., 2019). Sirtuin signaling which is inversely associated with Tau accumulation in AD, was differentially affected in retinal neuronal cells subjected to treatment with Aβ (Yin et al., 2018). Sirtuins protect cells via their inhibitory effects on NFκB signaling and accordingly, a negative correlation was observed between sirtuins and mTOR signaling in the retinal neurons upon treatment with Aβ (Guo et al., 2011; Mirzaei et al., 2019b). Furthermore, splicing factors such as Rp9, DHX38, and snRNP proteins like PRPF3 have been shown to play important roles in the retina (Lv et al., 2017; Ruzickova and Stanek, 2013).

Fig. 11. Biochemical pathway changes in AD mice retinas. (A) Functional interaction networks of differentially expressed proteins from 8-month old APP/PS1 mice retinas analyzed using open source Cytoscape software with the string DB plugin. The enriched networks are proteasomal enzymes, ribosomal machinery, and APP processing proteins. (B) List of proteins from interaction networks with corresponding fold changes. Reused with permission of Springer Nature for Mol Neurobiology, 2019, 56, pages 6017–6034 (Mirzaei et al., 2019b) (red- up and green- downregulated proteins).
While exposure to Aβ resulted in significant perturbations in these proteins indicating spliceosome impairment and RNA splicing changes in retinal neurons (Mirzaei et al., 2019a), similar defects have previously been reported in AD (Bai et al., 2013; Nuzzo et al., 2017).

Significant perturbations in the proteins associated with the actin cytoskeletal and keratin filament networks were also evident in the 661W retinal neuronal cells in response to Aβ treatment. Cytoskeletal assembly and cellular adaptability is affected in AD (Deng et al., 2019; Mirzaei et al., 2019b; Zempel and Mandelkow, 2012) and studies have shown that the sub-retinal administration of the Aβ42 peptide resulted in cytoskeletal disorganization in the retinal pigment epithelium cells (Bruban et al., 2009). Tau plays an important role in preserving neuronal cytoskeleton and axodendritic architecture and hyperphosphorylation of this protein promotes paired helical filament (PHF) and neurofibrillary tangle (NFT) formation, which orchestrates microtubular dysfunction and cytotoxicity. We observed elevated tau phosphorylation in 661W retinal cells in response to Aβ treatment, that also corresponded with GSK3β activation in these cells. GSK3β plays a major role in regulating tau phosphorylation and accordingly its pharmacological inactivation was recently shown to be neuroprotective and rescue behavioral and cognitive impairments in APP(SW)/Tau (VLW) mouse model of AD (Griebel et al., 2019). Thus, synergistic proteomics investigations that combine human post-mortem tissues with various animal and cellular models, will gradually unravel a true understanding of the retinal neurodegenerative alterations in various stages of AD pathogenesis.

5.2. Altered amyloid processing in the retina in AD

Various studies discussed above have demonstrated enhanced levels of Aβ and its parent protein APP in ageing and AD retinas, in both human and transgenic animal model tissues (Grimaldi et al., 2019;
Koronyo-Hamaoui et al., 2011; Mirzaei et al., 2019b). This is supported by recent findings that have demarcated differential abundance of amyloid proteins in various regions of the retina, with amyloid deposition particularly observed in the superior quadrant in human post-mortem tissues. Although, pathophysiological basis of these observations remains unclear, the findings were consistent with identification of amyloid positive spots in superior temporal and inferior temporal regions of the retina in AD patients in vivo (Koronyo et al., 2017).

Our proteomics screen identified a two to three-fold upregulation of APP and Aβ in the retinas of 2.5 month and 8-month-old APP/PS1 mice. Retinal neurons and glial cells also express other key proteins associated with AD pathology such as BACE1, γ-secretase, and ApoE, which are implicated in the metabolism of Aβ peptides in the brain (Sivak, 2013). In parallel to the APP changes, increased levels of APP processing enzymes were observed in animal retinas. Immunohistochemical staining revealed increased levels of APP, which was further validated by immunoblotting analysis of the retinal tissues (Mirzaei et al., 2019b). While 2.5 month APP/PS1 mice demonstrated only slightly elevated levels of small molecular weight Aβ peptides at 8 months, the parent APP levels were reduced, with a concomitant increase in Aβ and its oligomerized forms, illustrating altered APP processing with age in the retina (Fig. 12). This altered amyloid processing is supported by prior reports of Aβ plaque formation in cerebral cortex of mouse model starting at 6 weeks of age. Subsequently, amyloid deposition was identified in the hippocampus and other brain regions such as the thalamus, striatum, and brainstem between 3 and 5 months of age (Radde et al., 2006). By eight months, animals present with substantial cognitive and behavioral shortfalls, but without neuronal obliteration, which can camouflage bona fide changes in the protein expression (Cheng et al., 2013; Sadowski et al., 2004). APP staining was predominantly localized to the IPL and GCL, which supports the previous observations of inner retinal electrophysiological disturbances and thinning of retinal layers in APP/PS1 mice. This increased amyloid deposition in the vicinity of the INL and GCL correlated with increased RGC apoptosis in these mice at 13–16 months of age (Gupta et al., 2016b). Altered amyloid processing was also observed in human postmortem retinal tissues, wherein enhanced intracellular positivity for antibodies with APP affinity (6E10), in the absence of clear extracellular plaque formation was reported. These findings reflected intricate differences in the patterns of APP/Aβ processing and deposition between the retina and the brain (den Haan et al., 2018c).

Presenilin (PSEN1) is a transmembrane protein that comprises the catalytic subunit of the γ-secretase protease complex and is involved in APP processing. Although, while presenilin expression remained constant early on in life, it increased in AD mice as they aged to 8 months, suggesting the differential APP processing activity of the γ-secretase protease complex and concomitant changes in Aβ processing. Antibody staining of presenilin in the retinal sections revealed that the PSEN1 protein was predominantly localized to the inner retinal layers, which is the primary site of elevated Aβ immunoreactivity and also the region that shows the greatest extent of degenerative changes in the retinas of AD animal models (Mirzaei et al., 2019b) (Fig. 12). The observed changes in APP and Aβ levels, as well as its regulatory enzyme PSEN1, in the retina implicate that retinal imaging advances that could detect and improve AD diagnoses and track disease progression.

5.3. Activation of cellular proteolytic machinery in AD retinas

AD pathology can overwhelm the neuronal metabolism through accumulation of aggregating proteins. These misfolded and aggregated proteins are removed by activation of proteolytic enzymes and ubiquitin-proteasome system to maintain cellular proteostasis (Gadhave et al., 2016). APP/PS1 mouse model of AD exhibits enhanced proteolytic activation with age in retina and similar neurotoxic changes have been detected in 661W retinal neuronal cells upon exposure to Aβ (Deng et al., 2019; Mirzaei et al., 2019b). Plasmin—a potent proteolytic enzyme with broad specificity—is activated upon stimulation with Aβ peptide and has been implicated in mediating amyloid clearance from the tissues. The plasminogen system was shown to be able to act upon and degrade both monomeric and aggregated forms of Aβ peptide and protect primary cortical neurons from Aβ induced toxicity (Tucker et al., 2000). Increased plasmin activity is also reported in the glaucoma retina, where it was associated with reduced protease inhibitory activity of neuroserpin and increased digestion of extracellular matrix proteins in the optic nerve head region (Gupta et al., 2017). Another proteolytic enzyme, caspase-3 which is a key apoptosis mediator, is involved in expedited cleavage of APP and presenilin family of proteins that comprise γ-secretase protease complex. The enzyme has been shown to associate with the accumulation of intraneuronal Aβ42 peptides in 4 and 9 month old 5xFAD mice, thus contributing to ongoing neurodegenerative processes in AD pathology (Eimer and Vassar, 2013). Increased caspase-3 activation in 3x Tg-AD mice retinas was observed in advanced stages of AD (Grimaldi et al., 2018). Similar increase in cleaved caspase 3 staining was also detected in the human AD postmortem retinas and it co-localized with the RGCs (Grimaldi et al., 2019).

Retinal proteomics investigations have revealed an increased abundance of several key proteolytic enzymes and proteasome-associated proteins in the retinas of APP/PS1 mice. For example, cathepsin B—which plays a role in APP processing and regulation of Aβ levels—was specifically elevated in the GCL region in APP/PS1 mice (Mirzaei et al., 2019b). The modulation of cathepsin B activity has been shown to play a role in reducing Aβ production and in improving memory (Embry et al., 2017). Nicastrin—the largest constituent of the γ-secretase complex—similarly exhibits elevated expression within the inner retinal layers of APP/PS1 mice retinal tissues, as demonstrated by mass spectrometry and immunodetection analyses. It is a 709 amino acid type I transmembrane glycoprotein of 78 kDa, that binds to APP and plays key roles in Notch and Wnt signalling cascades. Using 5xFAD mice which express five AD related genetic variations; Nicastrin mediates the biochemical cascade of Aβ generation, and conditional inactivation of this protein led to reduced amyloid deposition (Sesele et al., 2013). Targeting this protein in the retina may lead to similar outcomes of reduced amyloid accumulation in AD.

Proteasome assemblies degrade distorted or aggregated proteins via proteolysis and play a key role in regulating protein quality control. Proteasome complexes are involved in regulating the proteolytic processing of aggregated Aβ and tau proteins. Aβ deposition could induce neurotoxic effects and promote unwarranted proteasomal activation in AD (Aso et al., 2012). Other reports suggest that protein catabolic processes, including proteasomal activity and ubiquitination, are diminished in AD brains. These varied effects can be indicative of various stages of the disease process or region- and tissue-specific effects. Our quantitative proteomics investigations revealed specific upregulation of proteasomal protein PSMB2 in the retinas of 8-month old APP/PS1 mice. PSMB2 is a critical component of 20S proteasomal complex and induces ATP-dependent cleavage of ubiquitinated proteins. Immunoblot analysis of the retinal tissues using anti-PSMB2 antibody did not show significant differences in expression of this protein. Therefore, we performed immunohistochemical analysis of the retinal sections which validated the proteomes findings and demonstrated striking upregulation of PSMB2 expression specifically in the GCL region (Mirzaei et al., 2019b) (Fig. 13). These findings signify that AD affects distinct populations of retinal cells in different ways and examining the whole retina may not necessarily be the best approach to identify disease specific variance. Future studies will elucidate whether proteasomal activation in AD retina is primarily caused by elevated Aβ and tau assemblies; or a cellular response to clear these proteins, or a combination of both processes.
5.4. AD crosstalk with protein biosynthesis, mitochondrial function, and cytoskeletal proteins in retina

The dysregulation of ribosomal function and protein biosynthesis are some of the initial biochemical processes that are affected in AD pathogenesis (Aso et al., 2012). Proteins involved in the mechanisms of transcription, translation, and elongation have been shown to be downregulated in APP/PS1 mice retinas using mass-spectrometry analysis. Wide-spread deviations in expression of various components of protein production machinery including initiation, translation, and elongation stages has been observed in ribosomes (Aso et al., 2012). Ribosomes are multi-component organelles that synthesize proteins in cells, and both mitochondrial and cytoplasmic fractions of ribosomal proteins are differentially affected in APP/PS1 mice retinas. We observed that 39S ribosomal protein L2 (MRPL2) which is a component of mitochondrial large ribosomal subunit, was reduced in the inner retinal layers (Fig. 13). The expression of elongation factor Eef1e1 also revealed downregulation in the inner retinal layers of APP/PS1 mice as a consequence of developing disease pathology (Mirzaei et al., 2019b) (Fig. 13). This is supported by evidence that upregulation of APP proteins correlates with negative modulation of protein synthesis and elongation processes. Tau hyperphosphorylation has similarly been suggested to promote ribosomal anomalies and suppress protein synthesis machinery, which may lead to the impairment of synaptic function and neural plasticity. Interestingly, in a proteomics study carried out by our group, we identified differential modulation of the abundance of seven mitochondrial ribosomal proteins in human glaucoma retinas, suggesting a potential biochemical overlap of pathological processes between the two neurodegenerative disorders (Mirzaei et al., 2017, Mirzaei et al., 2020b).

The accumulation of retinal Aβ in transgenic animals and the intraocular administration of this peptide aggravated wide-ranging neurotoxicity that is consistent with the biochemical pathways affected in ageing and disease conditions (Gupta et al., 2014b; Mirzaei et al., 2017). In 661W retinal neuronal cells, Aβ treatment had a significant impact on ribosomal protein synthesis, oxidative phosphorylation, cytoskeletal dynamics as well as spliceosome and lysosomal regulation. Indeed, similar to APP/PS1 mice retinas, treatment of retinal neuronal cells with Aβ42 peptide induced a decrease in mitochondrial and cytoplasmic ribosomal proteins following 6 h of Aβ treatment. A particularly strong downregulation of expression was observed for Rpl29 and Rps19 ribosomal proteins (Deng et al., 2019).

Progressive mitochondrial diminution has been consistently reported in AD models, with Aβ localizing at the mitochondrial membrane, which presumably chaperones cellular neurotoxicity through its inhibitory effects on pre-protein maturation (Cenini et al., 2016). Abnormal inter-neuronal Aβ accumulation may elicit mitochondrial dysfunction, which ultimately precedes cell loss (Williams et al., 2013). Mitochondrial markers such as opa1, dynamin 1 and mitofusin 1/2 are reduced in AD leading to alterations in mitochondrial fission and fusion processes. These processes protect the cells against stress by maintaining mitochondrial integrity, through both complementation and elimination of damaged organelles. Electron microscopic analysis indeed revealed a marked shift in the mitochondrial morphology and reduced mitochondrial integrity in the RGCs, in AD transgenic mice. These changes were accompanied by alterations in the mitochondrial cristae leading to enlarged inter-mitochondrial spaces alongside mitochondrial swelling in the RGC dendrites (Williams et al., 2013). The expression of several proteins associated with oxidative phosphorylation and mitochondrial function in retinal neuronal cells was differentially modulated upon treatment with Aβ. Ndufs4 and Atp6v1g1 were prominently downregulated (Deng et al., 2019), while abundance of several other proteins as a result of Aβ treatment corroborated the previous observations in AD pathology (Mirzaei et al., 2019b). Defects in Aβ and tau protein...
expression impinge upon mitochondrial integrity and synchronically promote vulnerability of oxidative phosphorylation machinery in animal models of AD (Williams et al., 2013). The accumulation of these proteins may preferentially affect RGCs due to their high energy requirements as indicated by mitochondrial enrichment in the dendrites and the fact that their axonal projections are unmyelinated until they enter lamina cribrosa (Casson et al., 2020). Lamina cribrosa is a perforated mesh-like structure where RGC axons converge and the tissue provides mechanical support against strain caused by transmigratory pressure gradients. Impaired mitochondrial integrity has been shown to alter energy metabolism and trigger early AD pathology, emphasizing the key involvement of mitochondrial dysfunction in AD pathology (Moreira et al., 2010).

Approximately 10 proteins associated with spliceosome and mRNA were also observed to be differentially expressed in response to Aβ treatment, suggesting that these processes are affected in response to Aβ exposure. For example, RNA binding protein Rbmx1l illustrated most noticeable upregulation of protein expression, with pre-mRNA splicing factor Isy1 exhibiting downregulation upon treatment with Aβ. Furthermore, while the RNA binding motif protein Rbmx was initially unchanged, its levels increased significantly as duration of Aβ incubation with retinal neuronal cells was extended to 24 h (Deng et al., 2019).

Cytoskeletal architecture—with twenty-eight actin microfilament and six keratin intermediate filament proteins—was differentially affected upon exposure to Aβ, suggesting that cellular stabilization, resistance to deformation and cell-matrix crosstalk are affected in retinal cells. Other proteins linked to the cytoskeletal system such as Tropomodulin 2, Murc, and Pfn2, were also differentially modulated in response to Aβ exposure (Deng et al., 2019). Tau microtubule-associated protein is involved in cellular signaling and serve neuroprotective functions, and tau hyperphosphorylation can lead to pathological self-assembly in AD. Augmentation of Ser202/Thr205 tau phosphorylation was detected in response to Aβ treatment in retinal neurons, with a parallel increase in glycogen synthase kinase 3β (GSK3β) phosphorylation (Deng et al., 2019). GSK3β interacts with tau and plays a role in axon formation, and its dysregulation triggers both Aβ production and a cascade of toxicity effects that lead to neuronal death in AD and cellular ageing (Chu et al., 2017). Together, these protein changes indicate that the toxic effects of Aβ on retinal neurons may be exacerbated through its modulatory effects on cytoskeletal organization and the filamentous architecture of the cells. A schematic representation of various potential molecular changes that are affected in the retina in AD is illustrated in Fig. 14.

5.5. BDNF/TrkB signaling in the retina in AD

Brain-derived neurotrophic factor (BDNF) is involved in mediating neurite outgrowth from RGCs, and can protect against optic nerve axonal degeneration and RGC apoptosis, as has been demonstrated in different experimental RGC injury models (Chitranshi et al., 2019b). BDNF is synthesized in both RGCs and Muller glial cells in the retina, and is also transported retrogradely from the superior colliculus and lateral geniculate nucleus to the RGCs via the optic nerve axons (Mysona et al., 2017). The protective effects of BDNF are accomplished via activation of downstream neuroprotective signaling such as PI3K/Akt/mTOR and MAPK/Erk 1/2 pathways through its high affinity receptor, tropomysin receptor kinase B (TrkB), which is also well expressed in the RGCs and optic nerve axons (Gupta et al., 2014a, 2014b). TrkB is a tyrosine kinase receptor that has high affinity for BDNF and is extensively involved in regulation of neuronal survival and mediating dendritic growth, synapse formation and neural plasticity in CNS (Chitranshi et al., 2018). Our studies have demonstrated that TrkB interacts with non-receptor tyrosine phosphatase Shp2 (PTPN11) in the RGCs in a caveolin dependent manner. Interaction with Shp2 leads to dephosphorylation of intracellular tyrosine kinase domain of TrkB and negatively modulates receptor activity (Abbasi et al., 2018, 2020; Gupta et al., 2012). Accordingly, we observed that Shp2 upregulation in the retina using AAV2 intravitreal administration resulted in loss of BDNF/TrkB signaling. This Shp2 modulation was also associated with upregulation of ER stress markers CHOP/GADD153, GRP78, p-PERK and apoptotic activation in the retina. AAV mediated Shp2 silencing using shRNAmir on the other hand, protected the RGCs against ER stress activation and apoptotic changes and rescued the BDNF/TrkB signaling under glaucomatous stress conditions (Chitranshi et al., 2017, 2019b). Exposure of SHSY5Y cells to Aβ peptide also eventuated the activation of GADD153 and pPERK markers and increased the expression of the pro-apoptotic BAD protein (Dheer et al., 2018).

Recent studies have described the loss of BDNF signaling in the AD

![Fig. 14. Schematic representation illustrating various molecular changes in the retina in AD conditions. Chronic neuronal damage induces activation of microglial cells that can generate pro-inflammatory molecules such as iNOS, NF-κB, TNFα, MHC-II, ROS and various cytokines (Haque et al., 2018) which can potentially affect neurogenesis and axonal remodeling.](image)
Brain and the association of BDNF Val66Met and Cys270Thr variants with cognitive changes and a heightened risk of developing AD (Shen et al., 2018). BDNF impairment has been shown to induce Aβ1–42 build-up in cases of AD and likewise, our studies have shown that BDNF impairment results in progressive accumulation of Aβ42 in the retina with age (Fig. 15), leading to a loss of neurons in the GCL and a decline in pSTR amplitudes (Gupta et al., 2014b). The induction of experimental glaucoma further exacerbated the retinal Aβ accumulation phenotype in BDNF heterozygous mice, suggesting that BDNF actions could be involved in the generation of Aβ or its degradation in the neural tissues (Gupta et al., 2014b). Similar to the retina, the sequestration of BDNF using anti-BDNF antibodies led to augmented amyloidogenesis in hippocampal neurons and accelerated senescence (Prakash and Kumar, 2014). Interestingly, the exogenous administration of BDNF to the hippocampus resulted in a reduction in Aβ levels in the tissue, which supports a key role for this neurotrophic factor in imparting protection against Aβ pathology (Zhou et al., 2008). The protective effect of BDNF are also evident from observations that BDNF infusion in the brain was not only effective in protecting the neurons but reversed the neuronal loss and synaptic deterioration associated with AD in an animal model. Further, while loss of BDNF has been shown to promote Aβ deposition in the inner retina and optic nerve, it is equally possible that increased Aβ deposition could restrict the retrograde transport of BDNF/Treb to the retina from the superior colliculus and lateral geniculate nucleus via optic nerve axons.

5.6. ApoE genetic polymorphism and its effects

Studies on multiple genetic and blood biomarkers have revealed a tight association between ApoE and the sporadic manifestation of AD (Gupta et al., 2011; Liu et al., 2013). The gene is mainly expressed as three different isoforms-ApoE2, ApoE3 and ApoE4 and the 34 kDa ApoE protein plays key regulatory roles in lipid metabolism in blood. Polymorphisms in the ApoE gene strongly correlate with the risk of AD, and plasma levels of ApoE could serve as a potential disease biomarker (Gupta et al., 2011). In addition, the E4 isoform of ApoE which is present in approximately 10–15% of the population, bestows a three-fold heightened risk of developing AD with representation in almost 50% of the AD patients. Having two copies of the E4 allele can increase the risk of developing AD by as much as 10–15 times (Michaelson, 2014). In contrast, the ApoE2 allele, which is relatively rare and is present in approximately 5% of the population, has been shown to reduce the risk of AD. Intriguingly, in comparison to the more widely-distributed ApoE3 allele which is present in 75–80% people and generally does not influence risk, the ApoE2 allele has been shown to increase the risk of developing AMD, while the ApoE4 allele diminishes this risk (Xiyue et al., 2017). The underlying mechanism for these puzzling differences involving reverse association of ApoE4 and E2 alleles with AD and AMD are not completely known. Subretinal inflammatory changes in AMD mouse model was shown to be ApoE isoform dependent, suggesting that the ApoE2 isoform is a risk factor and ApoE4 as beneficial in AMD (Levy et al., 2015). Further, retinoid X receptor (RXR) agonists have been implicated in mediating clearance of Aβ from the tissues by promoting ApoE levels. Our studies revealed that RXR agonist bexarotene reduced Aβ generation in SHSY5Y cells protecting them against ER stress and apoptosis (Chitranshi et al., 2019a; Dheer et al., 2018) and also protected the RGCs and optic nerve axons in animal models of experimental glaucoma (Dheer et al., 2019). In addition to ApoE, other major genetic polymorphisms that may or may not translate to protein sequence changes such as CHAT, CYP2D6, ABCA1, CHRNA7, ESRI, SNAP-25 etc. have been implicated in regulating the AD risk and responsiveness to various therapies (Sumiranturum et al., 2019). Future studies will unravel the correlation of ApoE and other genetic variants with ocular manifestations of disease. Ophthalmic investigations in association with genetic risk profiling could greatly improve AD diagnosis in early stages, evaluate disease course and clinically differentiate AD signs from other ocular disorders and age-related artefacts.

6. Neuroinflammation in the retina in AD

6.1. Neuroinflammatory biomarker changes in AD retinas

Chronic inflammation has increasingly been acknowledged as a hallmark feature of several age-related neurodegenerative disorders (Kinney et al., 2018). Accruing evidence indicates that sustained inflammation may compromise the core protective mechanisms that underlie neural injury in AD pathology (Heneka et al., 2015). Amyloid β plaques and tau protein PHFs and NFTs are often coupled with activated astrocytes and microglial cells in the AD brain (Selkoe and Hardy, 2016). Aβ and tau accumulation can lead to inflammatory reactions that mediate astrocytic and microglial activation and may trigger the release of inflammatory mediators such as cytokines and thus promote apoptosis in both the retina and brain tissues (Laurent et al., 2018). Astrogliosis is particularly evident in the GCL and inner and outer plexiform layers as evident by increased GFAP staining in retinal tissues from human AD subjects (Fig. 16). Pro-inflammatory molecules released by the microglia have been linked to the NLRP3 inflammasome and complement pathway activation (Spangenberg et al., 2016; Yue et al., 2017). It is well known that general neuroglial activation occurs with normal ageing; however, a significant increase in microglial cell density Fig. 15. BDNF loss promotes amyloid β accumulation in mouse retina with age. (A) Aβ1–42 levels were not affected in the optic nerve head of BDNF−/− mice at 2 and 5 months of age but were significantly increased at 1 year, compared to the WT animals as detected by ELISA (n = 5). Increased levels of soluble Aβ42 peptide were also detected in the optic nerve tissue of (B) WT mice exposed to microbead induced increased IOP of 23.71 ± 3.78 mm Hg over a period of 8 weeks. (C) Human post-mortem optic nerve tissues also revealed increased Aβ42 levels compared to the control tissues (n = 4 each). Data replotted with permission from (Gupta et al., 2014b), https://doi.org/10.1016/j.bbadis.2014.05.026, conveyed through Copyright Clearance Center, Inc.
is observed in the inner retinal layers under conditions of AD, with increased Iba-1 staining particularly in IPL and OPL regions (Fig. 16). Iba-1 is a Ca$^{2+}$ binding microglial protein that is implicated in cytoskeletal remodeling and mediates cell migration. These observations emphasize that increased expression of IL-1β and augmentation of disease associated microglia are observed in the post-mortem retinal tissues of AD subjects, as well as in mouse models (Grimaldi et al., 2019; Ramirez et al., 2017). The enhanced expression of IL-1β was prominently localized to INL and co-stained with microglial marker Iba-1 in human AD retina (Grimaldi et al., 2019). In response to chronic injury or exposure to the oligomerized neurotoxic proteins, these microglia migrate to sites of cell injury, secrete pro-inflammatory molecules, endeavoring to endocytose debris and protein fibrils (Ramirez et al., 2017). This neuroglial activation and the associated inflammatory response is also believed to mediate synaptic loss and neuronal degeneration (Spangenberg et al., 2016). However, while IL-1β expression is elevated in the astrocytes in the brain of an AD mouse model (Rosenzweig et al., 2019), cytokine reactivity here, did not reveal colocalization with GFAP-positive cells in human AD retinas. In contrast, the extracellular matrix protein and cytokine, osteopontin (OPN), which interacts with multiple integrin receptors and plays a role in immune activation, including chemotaxis and cell activation—was enriched in

Fig. 16. Increased pro-inflammatory markers in the post-mortem AD retinas. Representative images from control and AD retinas stained for (A) GFAP (red) and quantification of GFAP area covered by fluorescent signal/field of view n = 44/6 fields/patients (B) Iba1 (red) and quantification of its area shown by fluorescent signal/field of view n = 73/10 fields/patients (C) complement C3 (green) immunoreactivity. Red- GFAP and quantification of GFAP/C3 co-localization area/field of view, n = 45/6 fields/patients. Reused from (Grimaldi et al., 2019) under the Creative Commons Attribution 4.0 International License: Creativecommons.org/licenses/by/4.0/. Blue- Hoechst. Scale 20 μm. IL, inner layer; OL, outer layer.
AD retinas and colocalized with βIII tubulin in RGCs (Grimaldi et al., 2019). OPN is increased in AD brain tissues and treatments with immunomodulating compound glatiramer acetate (GA) resulted in extensive increase in OPN levels that corresponded with a decline in Aβ deposits in the brains of AD mice (Rentendorj et al., 2018).

Equally important is the role of TREM2 in CNS physiology, which has been widely recognized in recent years; as genetic variations in TREM2 enhance the risks of developing neurodegenerative diseases, and a surge in TREM2 transcript levels has been reported in AD brains (Sims et al., 2017). TREM2 receptor is shown to be expressed on microglial and dendritic cells and the molecule is now considered to be a crucial regulator of Aβ and tau levels via phagocytosis and plays a role in promoting microglial responses to their accumulation in AD, suggesting that its expression could mirror AD progression (Lee et al., 2018). Notwithstanding the fact that there were no differences in TREM2 in the retinas of AD patients when analyzed with fluorescence using in situ hybridization, the overall number of cells expressing TREM2 was much higher in AD retinas compared to the control tissues (Grimaldi et al., 2019), emphasizing that TREM2 expression was indeed increased. Apart from the role of innate immune responses, the S1PR agonist FYT720 which exerts anti-inflammatory actions by reducing the infiltration of lymphocytes to CNS has been shown to protect the neurons in AD (Aytan et al., 2016). Our group has demonstrated that the compound also protects the RGCs in rodent models of experimental glaucoma (You et al., 2014). These findings indicate that targeting S1PR may be useful in imparting neuroprotection to the RGCs in AD and in potentially blocking chronic neuroinflammatory components associated with the disease. This underscores the importance of innate immunity and microglial activation in retinal neurodegenerative changes in AD.

6.2. Complement pathway protein changes in retina

The innate immune system and activation of the classical comple- ment pathway plays an important role in regulating normal CNS physiology and tissue reorganization in healthy ageing and AD conditions. Multiple reports that highlight the involvement of complement pathways in AD pathophysiology have generated immense interest in its modulation, in a bid to impart protection against neuronal loss and chronic inflammation. Defective synaptic remodeling has been associated with inconsistent connectivity along the retinogeniculate pathway (Hong and Chen, 2011). C1q, C3, and C4 proteins have been shown to exhibit significant increases in expression in the AD brain (Fonseca et al., 2011). Aβ stimulated the expression of C3 in astrocytes, which in turn suppressed Aβ endocytosis resulting in aggravated AD pathology. In human AD postmortem retina, C3 expression coincided with GFAP-positive cells in the GCL (Fig. 16) and astrocytic activation by C3 has been shown to accelerate the deterioration of synaptic connections in AD. Whether alterations in levels of complement proteins in the retina is a result of increased synthesis or is augmented owing to compromised blood-retinal barrier remains to be established. Enhanced C1q levels in the AD brain have also been shown to correlate with tau protein accumulation (Dejanovic et al., 2018). Intriguingly, the inhibition of the C3a receptor resulted in reduced tau pathology in AD mice (Litvinchuk et al., 2018). Future studies should aim to investigate whether targeting C1q or inhibiting C3a receptor may be an alternative approach to target retinal tau pathology in AD.

Additionally, the expression level of complement factor H, a key repressor of the complement cascade, was reduced in the retina and in the anteroposterior axis of the visual pathway along the superior colliculus and primary visual cortex. This corroborated its reciprocal relationship with Aβ1-42 in Tg2576 and 5xFAD AD mice models (Alexandrova et al., 2011). This report supports the notion that the suppression of the complement pathway in retina may be inversely related to manifestation of AD pathology. Therapeutically inhibiting the complement pathway was also effective in rescuing the retinal ganglion neurons and imparting protection against synaptic and dendritic disruption in experimental models of glaucoma (Bosco et al., 2018; Williams et al., 2016). The aggregation of Aβ has further been extensively reported in sub-retinal drusen deposits in AMD and in response to ageing, which could elicit chronic complement activation and the associated degenerative retinal changes (Mirzaei et al., 2020a; Yehoshua et al., 2014). A greater understanding of the relative contribution of different complement pathway proteins that are specific to the retinal changes in AD, will lead to the development of mechanism-based strategies to restrict inflammation and protect the retinal function by augmenting synaptic connectivity and dendritic arborization.

6.3. Reduced crystallin levels in the AD retinas

Crystallins are a family of heat-shock adapter proteins that play a role in neuroinflammatory processes in glial cells and protect them against the detrimental effects of misfolded proteins. Crystallins were identified as one of the most downregulated class of proteins in the retinal proteomics analysis of eight-month-old APP/PS1 mice (Mirzaei et al., 2019b). These proteins are involved in neural regeneration and reactive astrogliosis and protect neurons and their dendritic arbor morphology against the toxicity of abnormally folded proteins. Therefore, reduced crystallin levels could make the retinal neurons in AD susceptible to degenerative changes and senescence (Piri et al., 2013). A reduction in the expression of α-crystallin was observed mainly in the GCL region of APP/PS1 mice (Mirzaei et al., 2019b) (Fig. 17). This α-crystallin isoform has been shown to colocalize with Aβ aggregates and inhibit their fibrillation and is often part of PHFs and NFTs. Intriguingly, the overexpression of this protein corresponded with diminished tau phosphorylation in N2a neuroblastoma cells, implying that crystallin loss could also potentially exacerbate tau pathological changes in retinal neurons (Ecroyd and Curver, 2009). In addition to decreases in the α1-form, a significant loss of β2-crystallin expression in various layers of the retina in APP/PS1 model using immunofluorescence and proteomics analyses, established this protein as one of the most differentially regulated proteins in AD retina. In a similar manner, the expression of β3-crystallin was reduced as well, and collectively these findings imply that almost the whole family of these proteins is downregulated in the retina in AD pathology (Mirzaei et al., 2019b) (Fig. 17). Diminishing levels of various crystallin proteins and their transcripts have previously been described in RGCs under experimental glaucoma conditions (Piri et al., 2013). Proteomics investigations have also established reduced levels of various crystallin family members in the initial stages of IOP elevation in the retina. The fact that this trend was subsequently reversed as the disease progressed signifies the time-dependent effects of IOP on crystallin protein expression (Anders et al., 2017a). A potentially relevant finding is that the intravireal administration of β-crystallin B2 protected the RGCs and retinal nerve fiber layer in case of experimental glaucoma and supported axonal regeneration in optic nerve injury models (Anders et al., 2017b). In agreement with these observations, our recent studies in human postmortem retinal samples established significant downregulation of eleven crystallin family members in POAG (Mirzaei et al., 2017). The downregulation of crystallins in both glaucoma and AD may represent a potential biochemical overlap that underlies these two divergent neurodegenerative disorders. Modulating expression of individual crystallin isoforms in retina using AAV under specific promoters, will elucidate the protective effects of these proteins in AD-induced retinal degenerative changes, thereby advancing future therapeutic developments.

7. Primary pathology in retina versus the effect of retrograde degeneration from brain

Apart from neurotoxicity induced by the accumulation of AD hallmarks proteins and accompanying inflammatory changes, the retrograde spread of AD pathology from higher visual centers in the brain via optic nerve axons could contribute to retinal pathology. Aβ fibrillation and
plaque formation has been reported in the higher visual centers including in the superior colliculus and primary visual cortex of AD subjects (Aizenstein et al., 2008), and neurodegeneration is indeed known to spread from the brain to anatomically connected neural areas (You et al., 2019). Optic nerve potentially facilitates the vesicular transport and diffusion of APP and tau proteins along RGC axons (Kipher-Kauer et al., 2010). APP and tau are co-transported with microtubular assemblies via axons that are propelled by kinesin and dynein proteins (Lacovich et al., 2017), which can be a potential mechanism underlying augmentation of the levels of these hallmark proteins in the retina from the brain (Gibbons et al., 2019). However, amyloid and tau aggregation can impair the diffusion of neurotrophins and axonal transport along the microtubular lattice leading to secondary insults. This is coupled with recognition of neuronal loss in the suprachiasmatic nucleus, which is involved in regulating circadian rhythms and the mRGC loss reported in AD could potentially be attributed to the retrograde degenerative changes that arise from this region of the brain (Zhou et al., 2016). Similar retrograde transmission of neurodegenerative changes to the retina has been demonstrated in MS pathology (Kiltroner et al., 2017). Meanwhile, glaucoma has been shown to propagate molecular and structural deficits in the higher visual centers through anterograde spread of degenerative changes that are believed to emanate primarily from the retina (You et al., 2019). If retrograde transsynaptic degeneration in AD plays a consequential role, neural changes in the higher visual centers in the brain are expected to topographically correlate with anatomically communicating regions of the retina. This can be investigated using combined brain and retinal imaging/functional studies, visual field assessment as well as through post-mortem analysis. This is of critical importance since increasing evidence from AD animal models shows that Aβ/tau protein alterations in AD may manifest in the retina prior to the brain, and this suggests that retinal disparities are more likely to be a primary pathology in AD (Koronyo-Hamaoui et al., 2011; Mirzaei et al., 2019b). However, the potential of secondary contributions induced by brain changes cannot be underestimated.

The correlation of retrograde changes in the retina in response to brain pathology was recently investigated by Haan et al. (2019), where they studied retinal thinning in a cohort of posterior cortical atrophy (PCA) subjects (den Haan et al., 2019). PCA is an atypical form of AD in which visual cortex and associated areas are specifically involved in the AD pathological process leading to remarkable visual symptomatology (Crutch et al., 2017). A correlation analysis of visual rating scores for cortical atrophy as measured by MRI, with pRNFL and total macular thickness changes in OCT revealed that while cortical thickness was discriminative between AD and controls, the retinal changes were not. This suggested smaller effect size in the retinal tissue compared to the brain. Further a comparison of pRNFL and macular thickness changes in healthy control and early onset AD (EOAD) patients with evidence of amyloid pathology in the brain demonstrated that retinal changes in general correlated with the extent of parietal cortical atrophy, but did not discriminate EOAD cases from controls (den Haan et al., 2018a). These findings suggested a relationship of retinal changes with visual cortex but also indicated that these changes were independent of amyloid pathology in the brain and could be more a reflection of ageing effects. Longitudinal follow up studies with sub-layer analysis of different regions of the retina are likely to provide further insights into the retrograde effects of AD pathology in the retina.

8. Overlap of retinal AD changes from other ocular disorders

Several neurodegenerative conditions of the brain such as AD, MS, Huntington’s, and Parkinson’s diseases have manifestations in the eye that foreshadow or continue concurrently with CNS pathology (Gupta et al., 2016a; Lee et al., 2019; London et al., 2013). Usually, these diseases have distinct clinical features to facilitate a differential diagnosis. However, several molecular and pathological aspects of AD overlap with glaucoma and AMD, including the deposition of archetypal proteins Aβ and tau in the retina (Gupta et al., 2016a). This means detectable...
choroidal degeneration have been reported in both AD and AMD patients (Cunha et al., 2016a; Zhao et al., 2015). Interestingly, the administration of Aβ antibodies targeting the c-terminal of the peptide in a mouse model of macular degeneration was shown to be protective for RPE and the retinal ultrastructure by reducing Aβ levels (Ding et al., 2011). Parallel to this, vaccination against Aβ peptides resulted in reduced retinal Aβ plaque formation in Tg2576 AD model (Li et al., 2009). Furthermore, studies have demonstrated an association between Aβ deposition and hyper-phosphorylated tau with ganglion cell degeneration in glaucoma, whereby Aβ deposition has been shown to be more localized in the inner retinal layers in glaucomatous eyes. Reports also indicate a higher prevalence of glaucoma in AD and that both the diseases are associated with progressive neural and vascular degenerative changes including microbleeds (Bayer et al., 2002; Golzan et al., 2017; Gupta et al., 2014b). Animal and human postmortem studies indicate increased levels of neurotoxic Aβ and its aggregates in the inner retina which correlates with RGC apoptosis in cases of both glaucoma and AD (Gupta et al., 2014b). Apart from the effects of neurodegenerative disorders mentioned above, ageing remains a primary risk factor that induces prominent molecular, functional, and structural alterations in the retina in both healthy and disease states.

Ageing is associated with progressive thinning of retinal layers. Ultrastructural analysis has revealed histopathological thickening of the RPE-Bruch’s membrane region with concomitant loss of the fenestration of the choroidal small vessels (Ida et al., 2004). OCTA analysis has demonstrated a similar loss of choroidal capillary bed in ageing subjects (Camino et al., 2019). These age-related changes are invariably associated with enhanced accumulation of drusen and pigment deposits that may in turn give rise to chronic inflammation. Enhanced accumulation of amyloid β peptides in the photoreceptors, RPE and the adjoining capillaries along with subretinal macrophage build-up has been reported with age in both human tissues as well as in rodent models (Hoh et al., 2010). Similar to that observed in the brain, age associated changes in the retina thus exhibit an overlap with effects of AD and could influence the early detection of AD associated symptoms in the eyes.

Our studies revealed that this increase in retinal Aβ was more pronounced in BDNF impaired animals, suggesting a key role of BDNF in regulating Aβ deposition in the retina (Gupta et al., 2014b). Elevated Aβ levels were also detected in the inner retinal layers and optic nerve of DBA/2J mice that progressively develop glaucomatous changes in the retina with age. Similar to human AD post-mortem retinal tissues, human glaucoma retinas also exhibit increased Aβ levels, and this makes it important to understand the molecular similarities and differences in the way these two diseases affect the neural tissue (Gupta et al., 2014b) (Fig. 15). Retinal imaging has revealed differences in the thickness of pRNFL and macular GCL regions, as well as an augmented global loss volume in both AD and glaucoma, although these deficits are clearly more marked in glaucoma patients as the disease progresses (Wang et al., 2018). Other points of differentiation include greater propensity for superior RNFL losses in AD as opposed to inferior RNFL deficits in the early stages of glaucoma. A possible distinction is the increase in IPL thickness in early stages of AD/MCI prior to thinning as opposed to inner retinal thinning associated with glaucoma neurodegeneration. Similarly, electrophysiological parameters that predominantly represent ganglion cell function have been reported to be affected in both glaucoma and AD. Prior research has described degenerative changes in the inner retinal layers, including reduced electrophysiological responses, in both AD and experimental glaucoma animal models (Gupta et al., 2016b). In contrast, abnormalities in the retinal vascular architecture and blood flow, the loss of RGCs in foveal region as well as NFL and choroidal degeneration have been reported in both AD and AMD patients (Cunha et al., 2016a). Along with it, shared determinants of the
disregulation of the complement system suggest that the involvement of common inflammatory mechanisms, increased oxidative damage, and mitochondrial impairment are common features of both AMD and AD pathologies.

There are although, spatial differences within retina that can be used to further understand and demarcate pathological changes between these disorders. For instance, while AD retinas involve NFL and GCL losses along with protein aggregation pathology, which is reported to be more prevalent in the peripheral superior quadrant; glaucoma predominantly affects inner retina with optic nerve excavation and axonal damage. AMD patients in contrast, show deficits in the central visual field, which is at variance with inferior visual field deficits reported in AD (Berisha et al., 2007). AMD is typically associated with progressive photoreceptor and RPE degenerative changes, while AD and glaucoma predominantly affect the GCL andNFL (Golzan et al., 2017; Ohno-Matsui, 2011). The characteristic patterns of neural and functional loss in glaucoma and AMD are well recognized, usually with quite different characteristics. Importantly, pigment deposition and drusen agglomerates frequently occur in the retinas of elderly people and can exhibit a wide degree of variation across different populations. This can interfere with retinal imaging by generating significant background noise and auto-fluorescence that needs to be filtered.

Our quantitative proteomics studies have revealed that several of the biochemical pathways are affected in a similar manner in the retina in glaucoma and AD conditions. For instance, mitochondrial function and oxidative phosphorylation are affected in both glaucoma and AD (Almasieh et al., 2012; Mirzaei et al., 2017, 2019b) and this makes RGCs particularly vulnerable to degenerative changes due to their high metabolic activity. Studies have shown that treatment with ubiquinone, electron acceptor in complex 1 and 2 of mitochondria can protect RGCs in glaucoma. Conversely blocking dynamin-related protein 1 (Drp1) which is key component of mitochondrial fission process was also shown to protect the RGCs in glaucoma model (Kim et al., 2015). Mitochondrial targeting may thus have important implications in protecting RGCs against degenerative changes in AD.

To date, the dominant viewpoint is that there is molecular overlap between these degenerative processes, and they may commonly co-exist in the same individual. This limits our current investigative tools to differentiate structural, functional, or physiological/blood flow changes in these disorders in early stages, even though they have some characteristic features. It also raises a question of whether AD with glaucoma symptoms is a different AD variant? Nonetheless, it is tempting to speculate that co-existence of glaucoma in AD may serve as a possible lead to discover new pathophysiological processes in both diseases. Currently, our inability to detect retinal molecular neuropathological changes by imaging technologies, precludes definitive AD diagnoses and monitoring. Future imaging advances may provide more disease specific recognition.

9. Clinical trials involving retinal changes in AD

Investigations into retinal changes in AD patients, post-mortem samples and animal models has gradually paved way for clinical trials in the last few years. As of 4th July 2020, there are at least 19 currently recruiting or recently completed studies that are registered as clinical trials in ClinicalTrials.gov database (https://clinicaltrials.gov/). This registry is maintained by National Library of Medicine at the National Institute of Health (NIH), USA (Table 1). The listed studies are primarily focussed on investigating retinal amyloid deposition and different aspects of retinal and other ocular changes in MCI and AD subjects. The projected number of participants in most of these studies range from below one hundred to approximately two hundred on average, however some of the collaborative studies have much larger projected enrolment numbers. For instance, Janssen Research & Development sponsored trial (NCT02114372) based in UK aims to recruit 1137 subjects in their prospective cognitive health in
### Table 1

Ongoing and Completed Clinical Trials investigating effects of AD on retina and other ocular changes (Status: R-Recruiting; C-Completed; A- Active, Not Recruiting).

| Identifier/ Status | Title | Study Start | Study End | Interventions | Number | Sponsor/Collaborators/Location |
|---------------------|-------|-------------|-----------|---------------|--------|--------------------------------|
| NCT03420807 (R)    | Retinal Metabolic Imaging of Alzheimer Patient | Dec 2017 | Sep 2020 | Device: MHRC | 50     | Optima Diagnostics Inc.; Sunnybrook Health Sciences Centre; McGill University; Polytechnique Montreal, Canada. |
| NCT01555827 (C)    | Retinal Neurodegenerative Signs in Alzheimer’s Diseases | Mar 2012 | Jun 2014 | Ophthalmological examination & Questionnaire | 200    | University Hospital Bordeaux, France |
| NCT03466177 (R)    | Multimodal Retinal Imaging in the Detection and Follow-up of Alzheimer’s Disease | Apr 2018 | Dec 2020 | Non-invasive, multimodal retinal imaging; Cerebral imaging; Neuropsychiatric testing; Venous blood sampling | 90     | Universitaire Ziekenhuizen Leuven, Leuven, Belgium |
| NCT02360527 (C)    | Retinal Neurodegeneration in Type 2 Diabetes as Biomarker for Alzheimer’s Disease | Sep 2014 | Dec 2015 |  | 126    | Hospital Universitari Vall d’Hebron Research Institute, Barcelona, Spain |
| NCT03447613 (C)    | Retinal Nerve Fiber Layer Thickness and Cerebrospinal Fluid Aβ/Tau | Aug 2013 | Dec 2014 | Procedure: surgery | 86     | Shanghai 10th People’s Hospital; Massachusetts General Hospital |
| NCT02590255 (C)    | Telomerase Activator and Retinal Amyloid | Dec 2016 | Dec 2018 | Dietary Supplement: Cycloastragenol and Placebo | 48     | Chippewa Valley Eye Clinic, Wisconsin, USA; TA-Sciences, USA |
| NCT03319810 (C)    | Effect of IVIG on Cerebral and Retinal Amyloid in Mild Cognitive Impairment Due to Alzheimer Disease | Jan 2018 | Jul 2018 | Biological: Octagam 10% | 5      | Sutter Health, Sutter Neuroscience Medical Group, CA, USA |
| NCT03233646 (R)    | OCT in Mild Cognitive Impairment and Alzheimer’s Disease | Jul 2017 | Apr 2021 | Device: Retinal Imaging | 1000   | Duke University Medical Center, North Carolina, USA; Alzheimer’s Drug Discovery Foundation; University of Edinburgh, UK |
| NCT03761381 (R)    | OCT Angiography and NRAI in Dementia | Sept 2018 | Dec 2020 | Device: Optical Coherence Tomography; Device: Noninvasive Retinal Amyloid Imaging (NRAI) | 20     | Oregon Health & Science University, Oregon, USA |
| NCT01937221 (C)    | Novel Retinal Imaging Biomarkers in Early Alzheimer’s Disease | Sep 2013 | Jul 2017 | Other: spectral domain | 60     | Duke University Medical Center; Duke Institute for Brain Sciences, North Carolina, USA; Alzheimer’s Association; Duke-NUS Graduate Medical School. |
| NCT03859245 (R)    | Photobiomodulation & Ketogenic Diet for Treatment of Mid-periphery Retinal Disorders for Alzheimer’s Disease Prevention | Feb 2019 | Sep 2020 | Behavioral: Photobiomodulation; Behavioral: Ketogenic diet | 30     | Bristlecone Health, Inc.; University of Minnesota; Yankee Eye Clinic, MN, USA |
| NCT02663531 (R)    | Retinal Neuro-vascular Coupling in Patients with Neurodegenerative Disease | Sep 2016 | Dec 2020 | Device: DVA; Device: FDOCT; Device: Pattern ERG; Device: Optical Coherence Tomography | 150    | University Hospitals Case Medical Center, OH; MetroHealth Medical Center, OH; Case Western Reserve University; National Institute on Aging (NIA), USA |
| NCT00921297 (C)    | Cataract Removal and Alzheimer’s Disease | Jun 2009 | Mar 2017 | Procedure: Immediate Cataract Surgery | 122    | Centre for Addiction and Mental Health; Kensington Eye Institute; Brain Canada; Weston Brain Institute; GE Healthcare; Sunnybrook Health Sciences Centre; St. Michael’s Hospital; University Health Network Canada; Baycrest Health Sciences, Canada. |
| NCT02524405 (R)    | BEAM: Brain-Eye Amyloid Memory Study | Feb 2016 | Dec 2021 | Other: Pittsburgh Compound B | 345    | University Hospital; University Hospital; AP-HM, Marseille; CHU de Bordeaux – Pellegrin; CHU de Dijon; CHU de Lille; Hospices civils de Lyon; AP-HM, Marseille; CHU de Montpellier; AP-HP – Hospital LARIBOISIERE; CHU de Strasbourg, France; Ministry for Health and Solidarity, France. |
| NCT02264899 (A)    | MEMENTO-VASCular Components of Dementia | Nov 2014 | Dec 2020 | Other: in Memento-VASCOD | 350    | CHU d’Amiens; CHU de Bordeaux – Pellegrin; CHU de Dijon; CHU de Lille; Hospices civils de Lyon; AP-HM, Marseille; CHU de Montpellier; AP-HP – Hospital LARIBOISIERE; CHU de Strasbourg, France; Ministry for Health and Solidarity, France. |
| NCT02114372 (A)    | Cognitive Health in Ageing Register: Investigational, Observational and Trial Studies in Dementia Research: Prospective Readiness Cohort Study | Feb 2014 | Apr 2023 |  | 1137   | Janssen Research & Development, LLC; Edinburgh, United Kingdom |
| NCT0160525 (C)     | The Eyes Have It: Ocular Saccade Abnormalities in Prodromal Alzheimer’s Disease | Dec 2012 | Dec 2015 | Other: Neuropsychological Assessment; Other: Ophthalmologic checkup; Other: Automated non-invasive ocularometry | 83     | University Hospital, Bordeaux; Lyon University Hospital; AP-HM, Marseille, France |
| NCT02141971 (C)    | Down Syndrome Biomarker Initiative (DSBI) | Jun 2013 | Apr 2017 |  | 12     | Michael Rafii; Janssen Research & Development, LLC; University of California, San Diego, USA |
| NCT04104373 (C)    | The Ontario Neurodegenerative Disease Research Initiative | Jul 2014 | Apr 2018 | Other: Observational Cohort | 522    | Ontario Neurodegeneration Disease Research Initiative; Hamilton Health Sciences Centre; Providence Care Mental Health Services; London Health Sciences Centre; Parkwood Institute; The Ottawa Hospital; University of Western Ontario, Canada. |

(continued on next page)
have been completed recently and therefore have not yet posted the outcomes from at least some of these studies. The outcomes from at least some of these trials will inform further research in the field including the need to incorporate measurement of retinal changes in various randomised AD drug trials. A relatively smaller study on “Retinal Metabolic Imaging of Alzheimer Patient” is sponsored by Optima Diagnostics (NCT03420807) which has a proposed enrolment of 50 subjects. Optima has developed a metabolic hyperspectral retinal camera (MHRC) and this trial seeks to differentiate MCI, AD and control subjects based on retinal hyperspectral signature using OCTA to assess structural and functional aspects of retinal microvasculature alterations in MCI and AD subjects. Thus, this research study is designed to provide a proof-of-concept for retinal microvascular changes as an effective screening tool in AD diagnosis. (Table 1).

The majority of these studies are in various stages of their progress or have been completed recently and therefore have not yet posted the results in public domain. The outcomes from at least some of these clinical trials will inform further research in the field including the need to incorporate measurement of retinal changes in various randomised AD drug trials. A relatively smaller study on “Retinal Metabolic Imaging of Alzheimer Patient” is sponsored by Optima Diagnostics (NCT03420807) which has a proposed enrolment of 50 subjects. Optima has developed a metabolic hyperspectral retinal camera (MHRC) and this trial seeks to differentiate MCI, AD and control subjects based on retinal hyperspectral signature using this instrument. This intervention study (Dec 2017–Sep 2020) is aimed to evaluate the feasibility of detection of Aβ using hyperspectral camera and detect specific autofluorescence or reflectance signature in the retina of AD subjects. A key objective of the study is to establish whether spectrally resolved Aβ deposition in the retina of AD subjects correlates with brain Aβ plaques as detected by 18F-flortaucipir amyloid PET scanning. Recently published data from this study revealed retinal vascular texture measure differences including higher mean venular tortuosity in 22 cognitively impaired subjects compared to a set of 26 cognitively normal people, in the spectral range of 450–550 nm (Sharafi et al., 2019). Differences in the retinal reflectance signature in AD subjects at around 550 nm were also reported independently in another cross-sectional study in a cohort of 35 participants comprising 15 MCI and 20 control subjects and the MHRC data was shown to correlate with brain amyloid PET scores (Hadox et al., 2019). More recently, Optima retinal imaging technology has achieved breakthrough device recognition from US FDA to support AD diagnosis and this designation is likely to expedite the development of the concept of hyperspectral imaging to identify specific changes in the retina in AD.

In addition to these ongoing and accomplished studies, there are at least 15 more studies listed in ClinicalTrials.gov registry which are either not yet recruiting or have since been withdrawn. For instance, the five-year longitudinal “Atlas of Retinal Imaging in Alzheimer’s Study” (ARIAS) (NCT03862222) sponsored by University of Rhode Island Red (Dec 2019 to Nov 2024) has a projected recruitment of 330 subjects. This study intends to examine the natural history of retinal imaging biomarkers associated with disease risk, disease burden, and progression in AD. A primary objective of this project is to create a reference database which can serve as gold standard for anatomical and functional imaging of the retina, in order to identify and develop sensitive and reliable markers of AD risk and/or progression. In addition, Moorfields Eye Hospital, UK has recently sponsored a three-year (Sep 2019–Aug 2022) retrospective study “Detecting Dementia in the Retina Using Optical Coherence Tomography” (NCT03631069). The investigators in this study have proposed to analyse repository of around one million OCT retinal scans, performed on patients since 2008, using various computational tools. This data will then be linked with other clinical data to establish whether the subjects were diagnosed with or went on to develop AD. A shortcoming of these clinical studies is that apart from significant heterogeneity amongst populations, different regions and cells of the retina can be affected differently. Therefore, it may not be possible to make a sweeping statement of the applicability of findings to all AD patients. More meaningful data can be generated by the design of longitudinal randomised controlled trials, which will however, require a significant collaborative effort between various vision and AD research groups and industry partners.

### Table 1 (continued)

| Identifier/ Status | Title Study | Study Start | Study End | Interventions | Number | Sponsor/Collaborators/Location |
|--------------------|-------------|-------------|-----------|---------------|--------|--------------------------------|
|                    |             |             |           |               |        |                                |

10. Conclusions, future directions and challenges

Recent research suggests that preclinical or quiescent stages of the disease might provide a critical window to initiate an effective therapy for the disease, which makes it critical to establish an early and reliable biomarker (Frisoni et al., 2017). The concept of early manifestation of AD related changes in the retina has evolved in recent years, which is supported by reports of alterations in AD prototypal proteins and accompanying neuronal injury and cell death in different regions of the retina. The clear optical path of the eye lends itself better for the detection of posterior segment changes at micrometer resolution, through visible and near infrared light-based ocular imaging modalities, and adaptive optics and functional OCT may take this to the next level. This provides a potential opportunity in AD biomarker research to identify and monitor the effects of AD on the retina, should future technologies be able to identify reproducible changes. The degeneration of RGCs and the corresponding axonal thinning of RNFL are key structural differences that have been reported in AD using OCT. Advances in OCT/OCTA have led to several reports of manifest correlations of retinal structural and vascular differences with the visual and cognitive vulnerability. This emphasizes the great utility of clinical imaging technologies in potentially assessing the subtle, yet discrete changes associated with various stages of AD. As technological advancements continue to gain momentum, we envision that well-designed long-term follow up studies will elucidate the clinical utility of monitoring retinal changes in AD patients. The trajectory of changes could then be used either alone or in combination with other measures to reach clinical decisions. For instance, retinal imaging modalities such as OCT may potentially be used in combination with blood...
bimer testing to screen at-risk populations. The positive patients can then be asked to undergo detailed brain imaging or CSF sampling to establish and monitor the disease process.

Pre-clinical research using animal models and human post-mortem studies have unraveled some of the novel mechanisms that underlie the disease process in the retinal tissues. For instance, increased APP levels, in the absence of typical brain-like extracellular Aβ plaque formation, could indicate discrete regulation of amyloidogenic and non-amyloidogenic pathways in the retina compared to the brain. Summarizing, despite the progress, a clear knowledge of the events that occur in the retina in various stages of AD pathophysiological process are lacking. Knowledge of these changes and comparing characteristic manifestations of the disease in brain, will enhance our understanding of AD neuropathology. In this review, we have endeavored to highlight several seminal studies that have enhanced our understanding of various aspects of amyloid and tau aggregation, structural and functional neurodegeneration, vascular changes and other molecular neuropathology of the retinal changes in AD. However, there are also several gaps in the field that impede establishing retinal changes as an AD biomarker and many questions remain unanswered, some of which are listed below:

✓ First, what is the extent of amyloid and tau protein aggregation in the retina in various stages of AD? Are these changes localized to specific types of cells or layers of the retina?

✓ Why are the retinal protein aggregates morphologically different from the brain deposits? Is it because retinal neural tissue is more resistant to AD pathology compared to the brain? Further, in contrast to the brain tissue, do amyloid and tau protein changes play a neutral, neurotoxic, or protective role in the retina.

✓ Determining whether retina is a primary site of AD pathology and establishing the contributions of retrograde degenerative changes in inducing retinal pathology.

✓ Although several groups are pursuing molecular imaging of retinal changes in AD using HSI, there is a dearth of longitudinal studies in well-defined cohorts, that can establish it as a meaningful clinical tool. Determining what molecular and structural factors may together contribute to these imaging differences remains a challenge.

✓ Apart from amyloid and tau, what are the other molecular, structural/vascular alterations that retina undergoes in early and later stages of the disease? This data will provide more comprehensive insights into the disease mechanisms. Whether any of the molecular retinal changes such as inflammation and apoptosis can be imaged in the retina with enough specificity and sensitivity, is an open question.

✓ What is the overlap of retinal AD changes with ageing and with other retinal disorders such as AMD and glaucoma? Recognizing the molecular differences, will help address key questions, such as what makes ApoE 4 and 2 genotypes to show opposite behavior in AD and AMD pathologies.

✓ Finally, can AD targeting drugs protect the retina against various changes in early and later stages of the disease.

The next few years may provide us exciting outcomes with more clarity on underlying mechanisms and the extent of retinal changes in AD. The field will have to simultaneously work on multiple fronts and correlate mechanistic knowledge from pre-clinical and postmortem studies with the clinical data to reach meaningful conclusions.

Limited availability of human ageing donor retinal tissues that are harvested immediately after death and stored appropriately, remains a challenge. The post-mortem retinal differences may be confounded by several unknown factors such as the existence of co-morbid neurodegenerative and other conditions as well as chronic exposure to therapeutic drugs while the subjects were alive. Thus, there is a need for a collaborative effort to develop a well-defined cohort of post-mortem ocular tissues from AD and pre-clinical AD subjects, that can be correlated with respective brain tissues and data. Further, both retinal structure and physiology depict considerable similarities between rodents and humans, and there is certain degree of neuropathological and behavioral recapitulation of AD symptoms in transgenic animal models. However, we need to be cognizant that most of the therapies such as semagacestat, solanezumab, verubecestat etc. that worked in AD mice models, ultimately failed to meet the primary endpoints in human trials, indicating fine pathophysiological differences between species (Egan et al., 2019; Gupta et al., 2013; Honig et al., 2018). These examples serve as salient reminders that transgenic animal models may not represent predominant sporadic form of AD pathology, and that establishing more relevant models is desirable. AAV mediated gene therapy is a fast-emerging approach that has the potential to be used in pre-clinical AD models to identify the disease mechanisms. Tissue specific inducible knock-out and overexpression mice models using CRISPR, cre/lox and AAV will provide distinct opportunities to study the roles of AD associated proteins in specific retinal cells. This will also allow performing rescue experiments and study the effects of retrograde/ante~dergative changes between the brain and the retina.

Finally, as mentioned above, our ability to image the AD-associated neuropathological remodeling that occurs in the retina and differentiating these changes from normal ageing process and other individual characteristics remains a foremost challenge, and in this regard, HSI appears to be a promising start. The ability to monitor molecular changes will provide a robust tool to explore the disease mechanisms and to monitor responses to interventions. In addition to monitoring amyloid deposition, future research should target the molecular imaging of tau phosphorylation, neuroinflammatory markers and early apoptotic changes in the AD retina for multipronged, tiered approach. Their use as retinal biomarkers in AD will be empowered by determining correlation of their longitudinal changes with neurocognitive and brain imaging pathologies, recognizing that certain ocular biomarkers might work better for diagnosis and others for staging. Correlating retinal changes with brain data will also help normalize inherent variability in longitudinal follow-up studies and establish the reliability of retinal functional and imaging tests in AD subjects. Future research will establish whether the distribution of Aβ build-up in the intracellular and extra-cellular retina compartments differs from that observed in brain tissue. Deciphering these mechanisms will unravel the extent to which retinal changes may serve as non-invasive AD biomarkers and will provide information regarding the susceptibility or resilience of specific neuronal populations to amyloid and tau pathology in the retina. In conclusion, our ability to comprehend the impact of AD on the retina will have broad implications in determining whether the eyes should be monitored more closely during AD progression. This will ultimately contribute to improved AD diagnosis and disease management.

Author statement

The authors declare that they do not have any known conflicts of interest.

Declaration of competing interest

The authors declare that they have no conflicts of interests.

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