



# One protein, multiple pathologies: multifaceted involvement of amyloid $\beta$ in neurodegenerative disorders of the brain and retina

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**Abstract** Accumulation of amyloid  $\beta$  (A $\beta$ ) and its aggregates in the ageing central nervous system is regarded synonymous to Alzheimer's disease (AD) pathology. Despite unquestionable advances in mechanistic and diagnostic aspects of the disease understanding, the primary cause of A $\beta$  accumulation as well as its in vivo roles remains elusive; nonetheless, the majority of the efforts to address pathological mechanisms for therapeutic development are focused towards moderating A $\beta$  accumulation in the brain. More recently, A $\beta$  deposition has been identified in the eye and is linked with distinct age-related diseases including age-related macular degeneration, glaucoma as well as AD. Awareness of the A $\beta$  accumulation in these markedly different degenerative disorders has led to an increasing body of work exploring overlapping mechanisms, a prospective biomarker role for A $\beta$  and the potential to use retina as a model for brain related neurodegenerative disorders. Here, we present an integrated view of current understanding of the retinal A $\beta$  deposition discussing the accumulation mechanisms, anticipated impacts and outlining ameliorative approaches that can be extrapolated to the retina for potential therapeutic benefits. Further longitudinal investigations in humans and animal models will determine retinal A $\beta$  association as a potential pathognomonic, diagnostic or prognostic biomarker.

**Keywords** Neurotrophic factors · Mitochondrial impairment · Protein aggregation · Inflammation · Oxidative stress · Electroretinogram · Optical coherence tomography · Metal ions · Vascular dysfunction

## Abbreviations

AD	Alzheimer's disease
AMD	Age-related macular degeneration
A $\beta$	Amyloid beta
RGC	Retinal ganglion cell
APP	Amyloid precursor protein
RNFL	Retinal nerve fibre layer
PERG	Pattern electroretinogram
VEP	Visual evoked potential
OCT	Optical coherence tomography
RPE	Retinal pigment epithelium
BrM	Bruch's membrane
IOP	Intra ocular pressure
BDNF	Brain derived neurotrophic factor
CNTF	Ciliary neurotrophic factor
GDNF	Glial derived neurotrophic factor
Arc	Activity regulated cytoskeleton-associated protein
BACE	$\beta$ Secretase
ACE	Angiotensin converting enzyme
AGE	Advanced glycation end product
VEGF	Vascular endothelial growth factor
INL	Inner nuclear layer
ROS	Reactive oxygen species
ONH	Optic nerve head
NSAID	Non-steroidal anti-inflammatory drugs
HRT	Hormone replacement therapy
LH	Luteinizing hormone

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GRH     Gonadotrophin releasing hormone  
SOD     Superoxide dismutase

## Introduction

The retina arises as a neuro-ectodermal derivative of the forebrain during development and delineates several physiological, cellular and biochemical similarities with the brain tissue. With the advancement in technology, subtle changes in both human and animal retinas can be directly imaged and assessed *in vivo* and as such the retina is being increasingly used as a model to study the neurodegenerative disorders of brain particularly Alzheimer's disease (AD) [1]. Additionally, retinal disorders such as age-related macular degeneration (AMD) and glaucoma are chronic neurodegenerative conditions that affect various retinal neurons and lead to progressive and irreversible loss of the vision. All three diseases are multifactorial with distinct pathological and clinical manifestations even though age remains the common primary risk factor and people with family history are at higher risk.

AD progressively ravages the brain and its cognitive ability and is accompanied by extreme cascade of pathological events marked by enhanced amyloid  $\beta$  ( $A\beta$ ) levels and its deposition in the central nervous system (CNS) [2]. Several human and animal studies have reported ocular changes in AD and pathological accumulation of  $A\beta$  in the retina [1, 3]. The prominent manifestation of glaucoma is increased intraocular pressure (IOP) with defining features of irreversible retinal ganglion cell (RGC) loss and damage to the optic nerve (ON) [4]. Human post-mortem and animal studies have established aggregation of  $A\beta$  and other proteins in the retina exposed to increased IOP which then promotes RGC apoptosis [5]. AMD, a leading cause of severe vision impairment in people aged over 60 years affects the macular region of the retina which is critical for the high resolution central vision. Multiple studies have identified  $A\beta$  deposition in the retina with AMD and its progression [6]. Although AMD and glaucoma are not classified as amyloidogenic diseases, the last decade has seen many animal and human studies featuring evidence of progressive accumulation of  $A\beta$  fragments in the retina [5]. Therefore, retinal  $A\beta$  accumulation can be regarded as a common feature of these three separate disorders (Fig. 1).

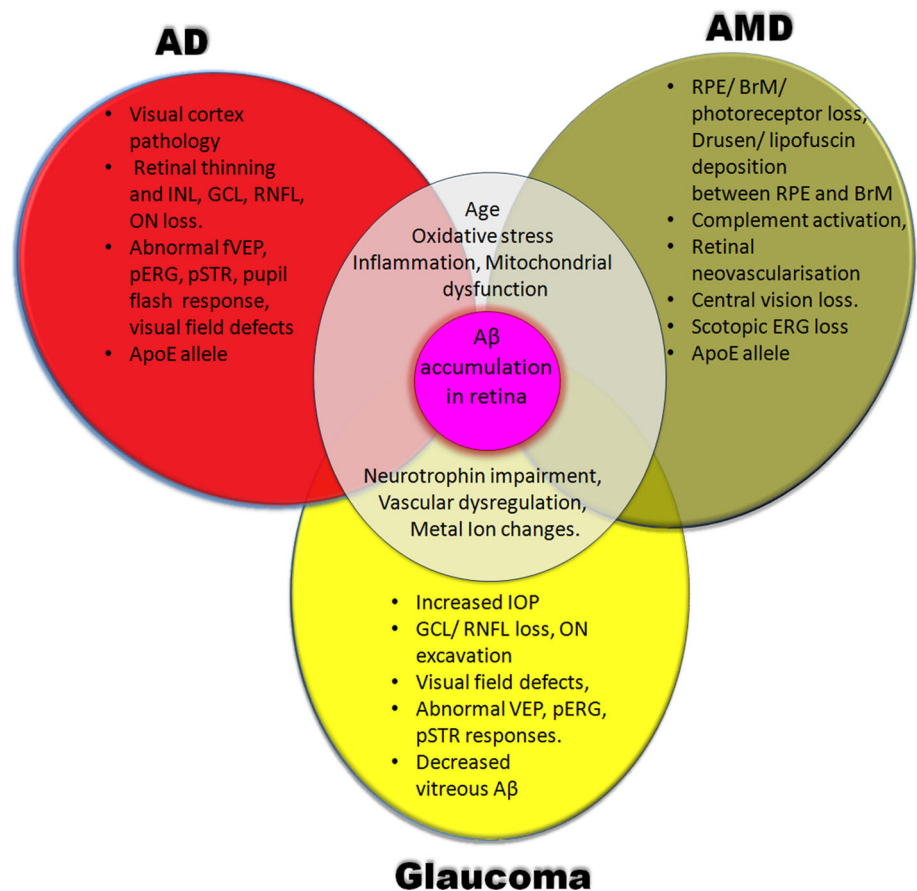
Amyloid precursor protein (APP) is expressed in various layers of the retina and plays roles in neurite outgrowth, synaptogenesis, and cell survival [7]. Sequential processing of APP generates various soluble and insoluble  $A\beta$  fragments including  $A\beta_{40}$  and  $A\beta_{42}$  [8]. Currently we cannot

fully explain the mechanisms underlying  $A\beta$  accumulation in the eye and higher visual centres hindering development of possible therapeutic options. Further investigations into retinal  $A\beta$  accumulation may be potentially insightful in terms of understanding neurodegenerative mechanisms as  $A\beta$  peptides have established neurotoxic properties and play a major role in inducing pathological changes in the AD brain [9, 10]. Several studies highlight the association of AD with inner retinal and ON degenerative changes which is reminiscent of glaucoma pathology. On the contrary, AMD primarily affects the outer retina with loss of photoreceptors and reduced RPE compliance [11].

A combination of several etiological factors including; excitotoxicity, oxidative stress, neuroinflammation and chronic gliosis, proteolytic degradation, dysregulation of ocular hemodynamic parameters and ischaemia, transsynaptic degenerative changes, genetic causes and aberrant cellular signalling are thought to be involved in neurodegeneration and cell loss associated with these disorders but the exact sequence of events or molecular basis of disease remains largely unknown [12, 13]. There is currently no treatment available to prevent neuro-degeneration associated with any of these disorders notwithstanding that these diseases together comprise a major global challenge across the personal, social and economic spectrum. Based on the epidemiological studies again, we do not have a clear answer as to whether incidence of cognitive impairment or AD is more common in AMD or glaucoma subjects or otherwise if the reverse of this true. While some studies found an association between either glaucoma or AMD and cognitive impairment [14, 15]; others showed no significant association [16, 17].

A greater understanding of the nature of link between these disorders will help in the identification of overlapping molecular mechanisms as well as development of novel and effective treatment strategies. Further research should aim to explain the origins of  $A\beta$  in the retina and determine whether  $A\beta$  is causative factor in these retinal diseases or is a downstream product of a primarily cortical disease or both. This will help elucidate whether treatment for AD may protect against retinal disorders or vice versa as well as establish whether retinal  $A\beta$  can be used as a diagnostic or prognostic biomarker. It will highlight the need for clinicians to monitor the retina in AD, especially when combined with either glaucoma or AMD. In this review, we discuss the potential causes and implications of  $A\beta$  aggregation in the retina in AD, AMD and glaucoma and touch upon possible therapeutic options for reducing ocular  $A\beta$  accumulation emphasising the pathophysiological similarities and differences between these diseases.

**Fig. 1** Schematic representation highlighting disease specific clinical and pathophysiological observations and their overlap in AD, AMD and glaucoma



## Retinal pathology associated with amyloid $\beta$ deposition

### Alzheimer's disease

Alzheimer's disease is associated with the accumulation of soluble A $\beta$  and its aggregated assemblies in the cerebral cortex and hippocampus leading to disrupted cellular metabolism, inflammation, neuronal cell death and cognitive decline [18]. A $\beta$  deposition in CNS also leads to development of sensory deficits in the olfactory and visual systems [1, 19]. Mounting evidence indicates A $\beta$  deposition in AD subjects and mice models corresponded to both retinal functional and structural deficits, reinforcing the concept of ocular pathological involvement in AD [1, 20]. We and others have shown congo red/thioflavin and antibody sensitive A $\beta$  deposition mainly localised to the inner part and photoreceptor layer of retina and optic disc in various mice models of AD. This is accompanied by RGC loss and decline in pSTR amplitudes suggesting the retinal specific origin of these deficits [1, 7, 21, 22]. Accordingly, Perez et al. observed that  $\sim 35\%$  of the A $\beta$  plaques were localised to the inner nuclear layer (INL) and recent studies highlight that there is a greater reduction in the thickness of

the INL and inner plexiform layers (IPL) of the retina [1, 21]. Rescue experiments carried out using A $\beta$  immunisation reduced A $\beta$  plaque formation in the retina and afforded protection against loss of retinal laminar structure providing further evidence that A $\beta$  plays a pathological role in the retina [22]. Our group and others have reported increase in TUNEL positive cells in the GCL highlighting enhanced apoptosis in the retina [1, 7]. Overall, neurotoxic effects of A $\beta$  causing persistent synaptic transmission defects, dendritic network remodelling of cholinergic amacrine cells and insufficient acetylcholine levels are suggested to underlie these pathological changes [21, 23]. However, altered expression or localisation of mutant APP and PS1 proteins in transgenic mice as potential basis of these effects cannot be disregarded. Investigating mice models with inducible mutant APP expression in the retina or specific subset of its neurons will help corroborate retina specific roles of A $\beta$ .

Clinically, AD subjects complain of frequent visual disturbances associated with colour discernment, stereoacuity, contrast consciousness, depth perception and comprehending structure from motion [24, 25]. A significant reduction of retinal nerve fibre layer (RNFL) thickness with loss initially occurring in the superior quadrant and

reduced inferior visual field sensitivity has been reported in early AD subjects [3, 24, 26]. Moreover, functional losses characterised by changes in pattern electroretinogram (PERG) and visual evoked potential (VEP) recordings demonstrate inner retinal involvement. Reduced ganglion cell layer (GCL) thickness and ON degeneration has also been observed in post-mortem AD retinas [25, 27]. Optical coherence tomography (OCT) identified a loss in RNFL thickness at the macula and regions surrounding the optic nerve head (ONH) [3, 6]. Besides retina, there is sufficient histological evidence to support that A $\beta$  deposits are formed in the visual cortex of AD subjects [28]. Thus apart from potential retinal A $\beta$  neurotoxicity and associated morbidity, it is possible that retinal pathological changes may be derived through trans-synaptic degenerative activity in the higher visual centres. Interestingly, A $\beta$  deposition has also been found in the lens leading to equatorial supranuclear cataract formation which bears a resemblance to the cataract formation in Down's syndrome patients, another disease involving A $\beta$  pathology [29]. Additionally, papillary abnormalities and altered pupil flash responses are observed in AD subjects [30]. A $\beta$  immunoreactivity is also reported in the lens and cornea of AD mice models substantiating the human observations [10].

Another clinically relevant aspect is recognising the timeline of A $\beta$  deposition in the retina relative to the AD brain pathology. Most animal studies show enhanced A $\beta$  deposition in the retina at a much later point than plaque formation is observed in the brain [7, 10, 22]. However, a recent report has claimed identifying A $\beta$  deposits in the APP/PS1 retinas well before the brain pathology [20]. While this may reflect different pathways of neuronal degeneration in APP/PS1 mice, biochemical and cellular changes in the retina may start well before A $\beta$  deposits are observed or disease diagnosis. Accordingly, several clinical studies have identified structural and functional retinal abnormalities even at early stages of cognitive impairment [3, 24].

### Age-related macular degeneration

Age-related macular degeneration is a progressive retinal degenerative disease influenced by both environmental and genetic factors. The disease is characterised by accumulation of cross-linked extracellular deposits collectively called drusen that form between the retinal pigment epithelium (RPE) and Bruch's membrane (BrM). Studies have reported A $\beta$  deposition in drusen particularly in the retinas of advanced AMD subjects [31]. Both A $\beta$ 40 and A $\beta$ 42 species have been identified in drusen and neo-vascular lesions in AMD retinas [11]. A $\beta$  deposits have also been identified in RPE proximity along with other histopathological changes and loss of scotopic

electroretinogram amplitudes [11]. Both soluble and oligomerised amyloid assemblies have been detected in drusen and RPE of human donor retinas [31]. Sub-retinal administration of A $\beta$ 42 in mice produced AMD-like symptoms manifested by decline in ERG amplitudes, RPE and photoreceptor degeneration including extensive vacuolation and BrM degeneration further highlighting its pathological involvement [32]. Collectively, these findings point towards a possible association of A $\beta$  with AMD pathogenesis.

The inheritance of the *APOE4* allele is associated with elevated cholesterol levels, which is a risk factor in AMD. Transgenic animals expressing human *ApoE4* exhibit a retinal phenotype that replicates many features of AMD and ERG loss associated with this model was attenuated by anti-A $\beta$  antibody treatment, strengthening the role for A $\beta$  toxicity in the AMD retina [11]. Interestingly, *ApoE* allelic variant is also a significant risk factor for sporadic type of late onset AD [33] and the commonalities between AMD and AD can be recognised in humans as well as in transgenic animals that express human *ApoE4* [6, 34]. *ApoE2*, *E3* and *ApoE4* have been shown to induce synaptic and ERG impairments in the retina of mice and *ApoE4* in particular is considered a susceptibility gene for AMD indicating overlapping molecular networks with AD [34]. While subjects with *ApoE4* are associated with greater risk of developing sporadic early and late type of AD deficits those with *ApoE2* are linked with lower risk [33, 35]. This is consistent with *ApoE* allele involvement in moderating A $\beta$  proteolysis and clearance, effects on A $\beta$  synaptic toxicity [36], reduced synaptic plasticity, increased neuroinflammation and increased cerebrovascular events in the brain in AD [33, 35].

Subjects with advanced AMD at baseline also showed an increased risk for developing AD [37]. AMD and AD share several clinical and pathological features, including underlying oxidative stress, inflammation and the formation of protein aggregates [38]. Accumulation of intracellular iron, lipofuscin deposits as well as mitochondrial and lysosomal dysfunctions seem to be common pathophysiological events in both AMD and AD [38]. Collectively, these observations suggest that the neuronal degeneration occurring in AMD and AD has at least a partial overlap of molecular mechanisms, despite these being two completely distinct diseases (Fig. 1).

### Glaucoma

Glaucoma is characterised by progressive damage to the RGCs and associated excavation of the ONH region. Abnormal APP metabolism and increased A $\beta$  expression in RGCs is associated with the development of RGC apoptosis in experimental models of glaucoma [5, 39]. A $\beta$  co-

localises with apoptotic RGCs and its administration induces significant RGC apoptosis in vivo in a quantity and time-dependent manner [39]. APP is also synthesised in RGCs and can be transported along the ON and synapses. Studies in human glaucoma tissues and rodent models of chronically elevated eye pressure indeed suggest disrupted axonal transport which may be linked to impairment of APP metabolism and contribute to upregulation of neurotoxic A $\beta$  in the retina [5, 40].

Exogenous intra-vitreous injections of A $\beta$ 42 and A $\beta$ 25–35 fragments significantly enhanced the RGC apoptosis, peaking at 3 days post-injection. Strong APP and A $\beta$  immunoreactivity especially close to the lamina cribrosa, GCL and ON has been detected and higher levels of A $\beta$ 40 are observed in the ON of old DBA/2 J mice [41]. Intra-vitreous administration of A $\beta$ 42 and A $\beta$ 25–35 peptides induced GC death and retinal degeneration in animal models [39]. Significant protection of the inner retina was achieved by A $\beta$  antibody as well as  $\beta$ -secretase inhibitor therapy [39].

It has been shown that targeting different components of the A $\beta$  formation and aggregation pathway can effectively reduce glaucomatous RGC apoptosis in animal models indicating that a combination of treatments may be more effective [39]. Our studies have identified enhanced levels of soluble A $\beta$ 42 in human ONH post-mortem tissues of glaucoma subjects [5] (Fig. 2a). A $\beta$  peptide is reported to be in the detectable range in the aqueous humour in 40 % of patients with glaucoma [42]. Decreased levels of A $\beta$  are reported in the vitreous of glaucoma patients [43] and although counterintuitive, this corresponds with the decreased CSF A $\beta$ 42 levels in AD patients. Interestingly, the impairment of brain-derived neurotrophic factor (BDNF), one of the key neurotrophic factors, has been

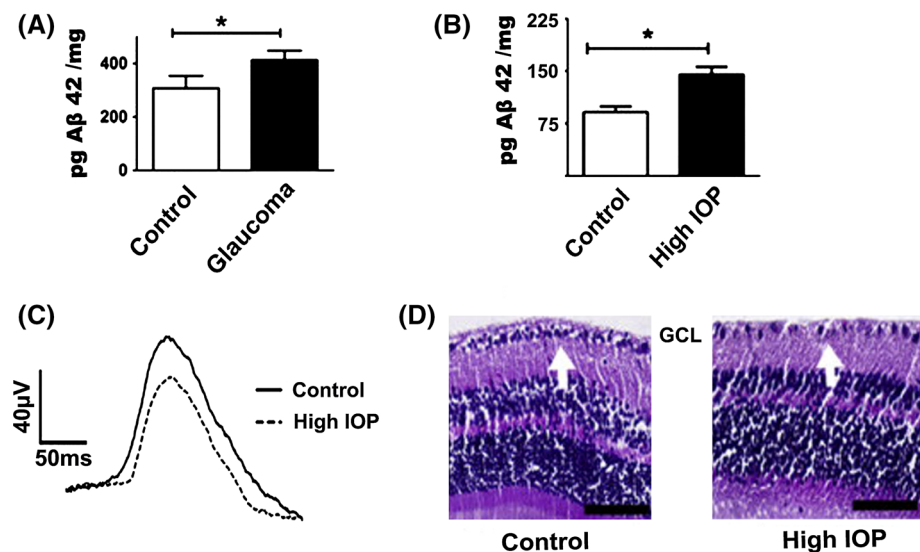
implicated in the pathogenesis of glaucoma and RGC damage. We observed enhanced levels of A $\beta$ 42 in the ONH regions of BDNF impaired animals exposed to increased IOP [5] (Fig. 2b).

Glaucoma prevalence is significantly higher in AD patients than in age-matched controls with the incidence found to be 5.2-fold in a German and 2.6-fold in a Japanese population [15, 44]. Evidence of a link between glaucoma and AD has also emerged from studies showing that patients with AD have RGC loss associated with prototypical glaucomatous changes, such as optic neuropathy and visual functional impairment [3, 27]. One study reported greater visual field defects in patients with open-angle glaucoma and AD vs patients with glaucoma alone [44]. However, larger prospective studies are needed to determine the extent of epidemiological links between the two diseases.

### Potential mechanisms of amyloid deposition in retina and its impact

Neurotoxic A $\beta$  aggregates cause synaptic degeneration and interfere with long term potentiation in the brain [45]. Changes in A $\beta$  levels can have detrimental effects on neuronal synaptic transmission of which the extent and exact mechanisms in the retina remain unknown. Similarly, whether the aggregation of A $\beta$  starts as a protective response in the retina or is a pathological phenomenon by itself is debatable. Further, different forms of A $\beta$  accumulation may have differential neurotoxicity. The potential mechanisms for A $\beta$  accumulation and its biochemical, structural and functional effects on the retina with reference to AD, AMD and glaucoma are discussed here.

**Fig. 2** Higher levels of soluble A $\beta$ 42 are observed in the **a**. ONH of human post-mortem glaucoma tissues ( $n = 4$ ;  $p < 0.01$ ) and **b** mouse ONH exposed to high IOP ( $n = 4$ ;  $p < 0.002$ ). **c** Loss of inner retinal function ( $n = 5$ ;  $p < 0.02$ ) and **d** reduced GCL density is observed in mice exposed to high IOP ( $n = 4$ ;  $p < 0.02$ ) [5]



## Metal ion and amyloid cross talk

There is much evidence to suggest that A $\beta$  accumulation is associated with disturbances in the equilibrium of redox active transition metal ions [2]. AD brains have increased concentrations of metals including iron [46] and aluminium [2] that catalyse the production of free radicals. Increased levels of divalent metal ions such as Cu and Zn are also implicated. Both Cu and Zn bind APP; and it is believed that this can modulate the functional properties of the molecule [47]. A $\beta$  precipitates out in the presence of these metal ions due to its high-affinity metal-binding site, and modulating this interaction can prevent the formation of A $\beta$  from APP. Studies have demonstrated high levels of Zn and Cu in the A $\beta$  plaques [47]. The potential significance of Zn in the aetiology of A $\beta$  formation is demonstrated by the fact that A $\beta$  deposition in APP transgenic mice is significantly reduced by breeding these with Zn transporter ablated animals that are unable to transport Zn into synaptic vesicles [48]. It would be interesting to determine whether retinas of these animals are protected against A $\beta$  accumulation when this model is overlaid with A $\beta$  associated retinal disease models. It is also worth noting that A $\beta$  plaques are concentrated in the most Zn rich area of the brain, the hippocampus, and levels of hippocampal Zn are elevated in AD brains than age-matched controls [47]. Importantly significant high concentrations of Zn are observed in drusen deposits in retina linked with AMD [49]. In contrast, Zn administration has been reported to be protective in dry form of AMD [50] which may indicate differential effects of Zn on various stages or forms of AMD. Alternatively, it is also possible that these effects are attributed to differential localisation of the metal concentrations in retina. Cumulative oxidative damage caused by divalent metal ions may exacerbate A $\beta$  neurotoxicity and lead to its enhanced aggregation and deposition [51].

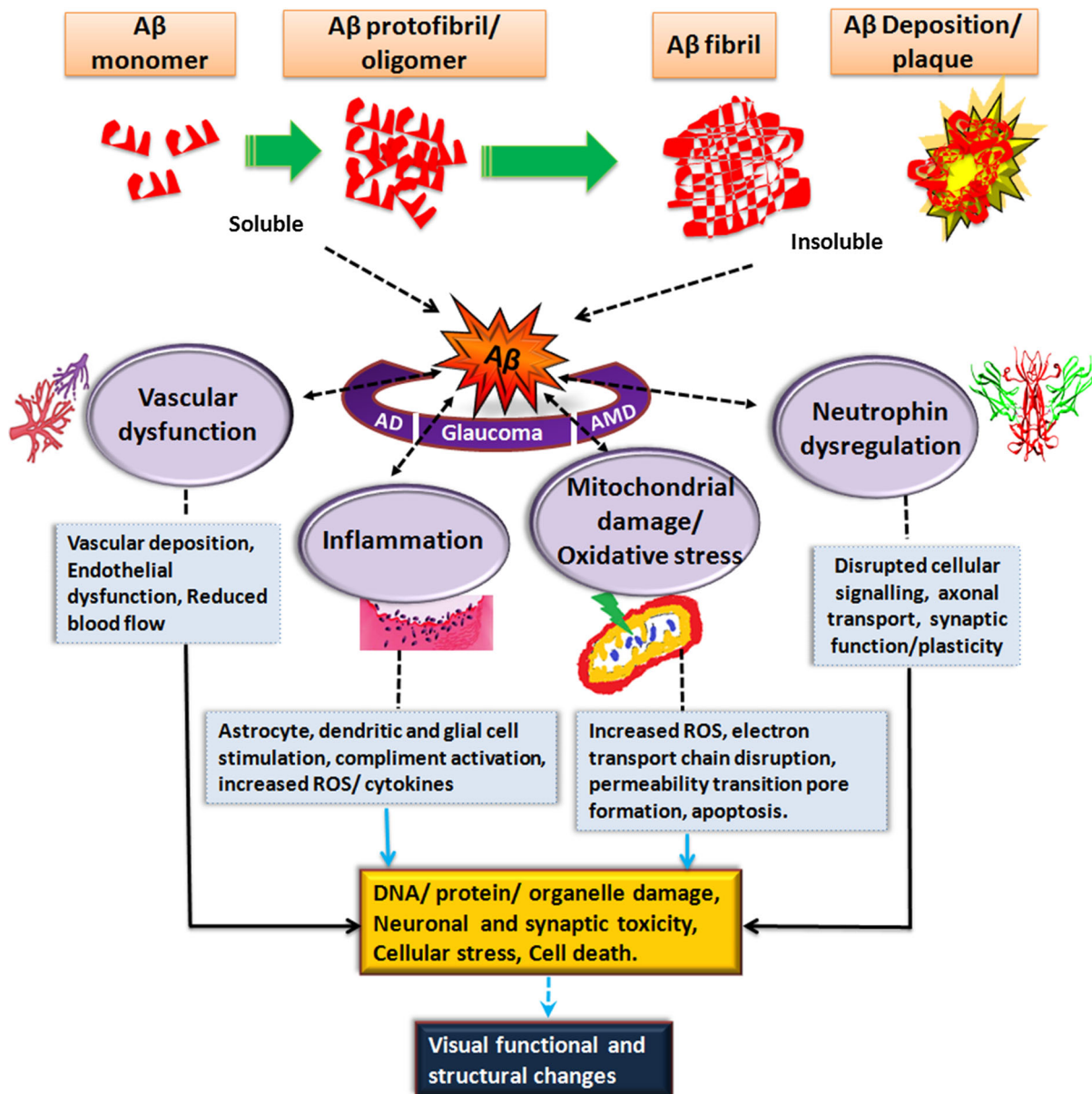
Interestingly, ceruloplasmin- and hephaestin-deficient mice with disrupted Cu and Fe transportation and metabolism exhibit retinal degenerative changes similar to that observed in AMD which may provide further mechanistic insights into the role of metal ions in retina [52]. The effect of metal ion dysregulation has not been well characterised in glaucoma, but mitochondrial impairment in the disease points towards local disturbances in catalytic role of metal ions involved in electron transport. The role of Cu may be important in this as it is critical for the activity of many enzymes such as cytochrome-c oxidase. Accumulation of non-heme iron promotes disruption of cellular homeostasis and contributes to the mitochondrial dysfunction in AD and an analogous role of metal ion in the retinal mitochondrial impairment cannot be ruled out [53]. Further studies will unravel the causal links of chronic metal ion

dysmetabolism in the retinal A $\beta$  accumulation associated with the glaucoma, AMD and AD.

## Neurotrophin impairment

Impairment of neurotrophins particularly BDNF and TrkB actions has been strongly suggested in AD and glaucoma pathologies while other neurotrophic factors like CNTF play an important role to protect the photoreceptors in AMD [5, 54]. Neurotrophins regulate Akt, Erk and Stat3 cellular signalling pathways which are critical for retinal neuroprotection in both AMD and glaucoma. Soluble forms of A $\beta$  are implicated in mediating reduced BDNF mRNA levels and compromised BDNF signalling in the neuronal cells [55]. A $\beta$ 42 levels and A $\beta$ 42/A $\beta$ 40 ratios strongly correlate with decreased BDNF levels in the brain and retina [5, 56]. A $\beta$  oligomerisation has also been demonstrated to impair BDNF retrograde transport along the axons [55] (Fig. 3). Briefly, A $\beta$  upregulation is associated with decrease in BDNF levels in neurons, and in the retinal milieu this may lead to neurodegenerative changes by disrupting axonal transport and synaptic function. Brain cortical and hippocampal areas which exhibit large-scale amyloid pathology in AD demonstrate downregulation of BDNF levels [56, 57]. Similar disruption of neurotrophic factor homeostasis in ON, retina or its specific regions in advanced stages of glaucoma, AD or AMD in response to A $\beta$  accumulation cannot be excluded. In the retina, our studies have shown an increase in soluble A $\beta$  in the aged BDNF<sup>+/-</sup> mice indicating that BDNF perturbations may be responsible for enhanced amyloidogenesis in the retina [5]. These observations correspond with the significant inner retinal structural and functional loss in BDNF<sup>+/-</sup> animals highlighting its important role (Fig. 2c, d). A $\beta$  levels were further exacerbated in the BDNF<sup>+/-</sup> mice exposed to experimental glaucoma and these changes commensurated with a reduced BDNF/TrkB signalling [5]. Decrease in BDNF levels and an increase in A $\beta$  is reported in various models of experimental glaucoma as well in post-mortem tissues from glaucoma subjects [5].

Increased A $\beta$ -related cognitive impairment has also been reported in individuals having BDNF Val<sup>66</sup>Met polymorphism along with the apolipoprotein *ApoE $\epsilon$ 4* allele in AD [58]. Although BDNF/TrkB signalling plays important roles in the retina and its impairment is associated with retinal A $\beta$  perturbations, association of BDNF SNPs in retinal disorders or in mediating A $\beta$  changes in the retina in AD and other retinal neurodegenerative disorders so far is speculative. Amyloid  $\beta$  aggregation may also lead to down-regulation of BDNF levels through sequestration, axonal transport obstruction and synaptic impairment [56]. This corresponds with the observations that A $\beta$  oligomerisation strongly inhibits the BDNF-induced



**Fig. 3** Flow chart illustrating the impact of  $A\beta$  on the visual structure and function in AD, AMD and glaucoma

increase of activity-regulated cytoskeleton-associated protein (Arc) which plays a role in synaptic plasticity [59]. Further, it has also been shown that  $A\beta$  exposure results in up-regulation of full-length TrkB expression and a down-regulation in the levels of truncated TrkB in the neuronal cells indicating differential effects on various receptor subtypes and consequently different effects on downstream signalling have been observed [60].

Similar to BDNF involvement, a causal link has been suggested between NGF imbalance and activation of the amyloidogenic pathway in AD [61]. Prolonged treatment with NGF is suggested to be beneficial in improving visual acuity and electrophysiological responses in AMD [62].

CNTF administration provided significant protection against high IOP induced RGC degeneration in animal model. It is already being tested in clinical trials for non-exudative AMD and reported to be effective in stabilising visual acuity [54, 63]. Given that CNTF treatment was effective in shielding against synaptic impairment and mitigating memory loss in mouse model of AD [64], it may have potential beneficial effects for the retina in AD. TrkA and TrkB activation imparts neuroprotection to the RGCs in experimental models of glaucoma [5, 65, 66] and  $A\beta$  binding to TrkB-FL receptor negatively affects the synaptic plasticity [67].  $A\beta$  binding to the p75NTR has similarly been demonstrated to induce apoptosis and p75NTR

suppression protected RGCs following ON injury indicating potential pathological roles of A $\beta$  in the retina [65, 68].

### Association with inflammatory pathways

A $\beta$  is associated with microglia, astrocyte and dendritic cell activation and is also an activator of the complement factors [69]. AD mice models depict significant upregulation of inflammatory markers in their retinas. Elevated GFAP and IBA1 levels were observed in various layers of Tg2576 mice retinas indicating astrocyte and microglial upregulation [22]. The APP/PS1 mouse model of AD also depicted increased levels of F4/80 macrophage marker in the retina indicating activation of microglial cells [21]. Astrocyte activation has been suggested to regulate ONH excavation and RNFL loss through the stimulation of matrix metalloproteases in glaucoma [70]. In contrast, AMD is a chronic inflammatory disease involving defective regulation of alternative complement pathway which generates inflammatory mediators. Drusen deposits are the major site of local chronic inflammation in AMD and A $\beta$  co-localises with activated complement components in drusen deposits [71, 72]. It is still not precisely known what triggers the complement cascade; however, A $\beta$  in drusen may be a candidate peptide, as it has a known role in promoting the complement pathways in AD [73]. Also, low levels of complementary factor H were observed in the retinas of various AD models suggesting a link of complement pathway dysregulation with amyloid deposition [74]. C1q which is the first element in the complement pathway exhibits increased neuronal expression both in AD as well as in animal models of glaucoma [75, 76].

Moreover, increased monocyte chemotactic protein (MCP1) staining in the GCL coinciding with sites of A $\beta$  deposition was observed in APP/PS1 animals along with TUNEL positive cells [7]. Inflammatory cytokines, TNF $\alpha$  and its receptors TNFR1 $\alpha$ / $\beta$  have also been noted to be upregulated in the retinas of glaucoma patients [77].  $\beta$  amyloid stimulates secretion of TNF $\alpha$  which stimulates iNOS production through sphingomyelinase pathway [78]. Interestingly, TNFR1 $\beta$  ablation affords robust neuroprotection to RGCs in mouse model of elevated IOP. Gene-profiling studies have also indicated upregulation of serum amyloid A which is an inflammatory marker in the trabecular meshwork and retina in human glaucoma [79]. Reduction in A $\beta$ -derived neuro-inflammation through minocycline treatment which leads to microglial inhibition has protective effects in AD as well as on the RGCs in glaucoma [6, 80]. Overall, increased glial number and inflammatory markers may contribute to degenerative changes in the retina by releasing cytokines and ROS, although these may also be playing a role in the elimination of A $\beta$  assemblies [81].

### Imbalance of proteolytic system in the retina

Physiological regulation of proteolytic processes is fundamental to both the generation and clearance of toxic A $\beta$  species.  $\alpha$ ,  $\beta$  and  $\gamma$ -secretase activity mediates generation of various A $\beta$  fragments and these enzymes are, thus, imperative focus to modulate A $\beta$  accumulation [82].  $\beta$ -secretase 1 (BACE1) in particular initiates the formation of  $\beta$ -amyloid, and the fact that BACE1 levels are elevated in the AD brain provide compelling reasons to develop therapies directed at BACE1 inhibition to reduce  $\beta$ -amyloid generation. BACE1 changes in the retina under pathological conditions are not known although treatment with  $\beta$ -secretase inhibitors exhibits a protective effect on the RGCs against damage caused by excitotoxicity. There is evidence of neuroprotective effects of BACE1 inhibition in experimental models of ON damage [83]. More recent studies, however, highlighted that BACE<sup>-/-</sup> mice illustrate retinal thinning, apoptosis, loss of retinal blood architecture and lipofuscin accumulation. Pharmacological inhibition of BACE1 also resulted in increased choroidal neo-vascularization in animals [84]. These along with observations made by Devi and Ohno that partial BACE1 suppression did not reduce cerebral A $\beta$  deposition in AD mice [85] suggest that targeting BACE1 might not be a valid strategy mainly due to associated side effects in the retina.

$\gamma$ -secretase, on the other hand, regulates VEGFR signalling in RPE as well as vascular permeability and angiogenesis in retinal endothelial cells [86]. It also affects endothelial function through its modulation of notch signalling [87]. Targeting  $\gamma$ -secretase–presenilin complex may therefore find applications in pathology associated with abnormal vascularisation in retina such as in wet AMD. Furthermore, A $\beta$  once generated is subject to proteolytic degradation by A $\beta$ -degrading proteases such as endothelin-converting enzyme (ECE) and insulin-degrading enzyme although not much is known about their roles in the retina. Extracellular A $\beta$  breakdown is accomplished mainly through metalloproteases like neprilysin and angiotensin converting enzyme (ACE). Interestingly, mice that lack neprilysin develop RPE degeneration, and exhibit sub-RPE A $\beta$  deposits similar to that observed in AMD in humans [88]. ACE is also expressed in retina and vitreous humour in human and animal tissues [89]. Activation of endogenous ACE2 has been suggested as a potential therapeutic strategy for glaucoma treatment [89]. Plasmin and its activators have also been shown to be both activated by and involved in the degradation of A $\beta$  aggregates [90]. Activation of plasminogen system mediates excitotoxicity induced injury to the RGCs and is associated with glaucomatous damage [91]. We have shown that A $\beta$  aggregates form complexes with the protease inhibitor neuroserpin in the retina [92] and this can potentially limit proteolytic



actions of plasminogen system on the  $A\beta$  assemblies and obstruct the clearance mechanisms.

### Lysosomal and autophagic dysregulation

There is reasonable evidence to support disturbances of the lysosomal vacuolar system and its hydrolytic enzymes associated with  $A\beta$  generation and neurotoxicity in AD [93].  $A\beta$  has been identified in various components of the lysosomal system, such as rab5 endosomes; autophagic vacuoles and multivesicular bodies. Several studies have recognised the endosomal/lysosomal pathway as an important regulator of APP processing. Lysosomal involvement in  $A\beta$  generation is further discernible as these organelles contain both APP as substrate and  $\beta$ - and  $\gamma$ -secretases as its processing enzymes [94]. Endosomes are involved in  $A\beta$  production from APP and mediate the uptake of  $A\beta$  and soluble APP.  $A\beta$  accumulates within neuronal lysosomes in AD mice brains and may undergo deposition in the retina by similar mechanisms [95]. Among a wide spectrum of lysosomal enzymes, cathepsins are associated with both production and degradation of  $A\beta$  directly as well as through activation of other proteases [96]. Cathepsins are well expressed in the retina and are upregulated in AMD and glaucoma conditions [97]. These changes reflect biochemical facilitation to increase the clearance of toxic and degradation products and can play crucial role in modulating  $A\beta$  levels in the retina. With the  $A\beta$  build-up in vacuolar structures, lysosomal dysfunction ensues and eventually the cellular degeneration contributes to discharge of  $A\beta$  and its oligomeric forms into the extracellular space.

Interestingly, inheritance of the *ApoE4* allele is reported to exacerbate endocytosis upregulation and potentiate  $A\beta$  induced lysosomal impairment [98]. More recent studies also support impaired lysosomal autophagy in RPE dysfunction with implications in AMD [99]. Lipofuscin and drusen accumulate within lysosomes in RPE along with  $A\beta$  and negatively affect phagocytosis of casted photoreceptor outer segments [38]. Autophagosomes were also observed to be increased in the GCL, 1–4 weeks after exposure to elevated IOP in a chronic model of experimental glaucoma. Upregulation of autophagy indicators LC3-II and p62 subsequent to IOP elevation suggests potential autophagic flux disturbances [100]. Autophagy activation is suggested to exert a protective after ON injury and protect RGCs under stress conditions [101].

### Mitochondrial dysfunction

Mitochondrial dysfunction is a prominent feature of  $A\beta$  induced neuronal toxicity in AD. APP and  $A\beta$  can cross the mitochondrial membrane, where they can interact with

mitochondrial components, obstruct the transport of proteins to mitochondria, disrupt the electron transport chain, increase ROS production and actuate mitochondrial damage leading to neuronal dysfunction [102].  $A\beta$  has been shown to promote formation of permeability transition pore in mitochondria and promote apoptosis [103]. Mitochondrial impairment makes less energy available to carry out the repair processes predisposing retinal cells to apoptosis [104].  $A\beta$  is also associated with modulation of mitochondrial  $Ca^{2+}$  levels leading to alterations in organelle physiology and enhancing self fragmentation [105]. Being metabolically highly active, aberrant mitochondrial function in photoreceptors and RPE can have a significant impact in AMD. Mitochondrial dysfunction and disruption of mitochondrial axonal transport is strongly suggested as a pathophysiological feature in glaucoma, which may disturb the local energy balance and increase susceptibility of RGC to degeneration. Inhibition of histone deacetylation can rescue the hippocampal neurons from  $A\beta$ -induced impairment of mitochondrial axonal transport. This inhibition also provides significant protection to RGCs in ON injury models [106]. Failure to meet the energy demands in axonal and dendritic terminals may eventually cause synaptic dysfunction in the retinal neurons. Elucidating the impact of  $A\beta$  accumulation on retinal mitochondria in AD, AMD and glaucoma may uncover novel therapeutic targets to help protect the retina.

### Oxidative stress

There is evidence supporting widespread oxidative stress triggered by increased ROS production and a dysfunctional antioxidant system in these three disorders [6] (Fig. 1). Prolonged exposure to increased oxidative stress is characterised by biochemical changes including lipid peroxidation, nitration, accumulation of advanced glycation end products (AGEs) and protein/nucleic acid damage [107]. In brain, oxidative stress markers may appear several years prior to significant  $A\beta$  deposition [107] and it is likely that similar changes occur in the retina in AD and other retinal disorders linked with  $A\beta$  accumulation. Oxidative stress is suggestively involved in the amyloidogenic processing of APP, thereby contributing to elevated intracellular  $A\beta$  levels [108].  $A\beta$  also tends to undergo increased aggregation in the presence of free radicals, which enhances pro-oxidative environment by generating more ROS advancing cellular injury.  $A\beta$  deposition may in turn further amplify the neurotoxic effects by increasing neuronal sensitivity to oxidative stress [108]. Epidemiologic, genetic, and biochemical studies support a role for oxidative stress in AMD pathogenesis and RGC and ON damage in glaucoma. The macular region is particularly exposed to the high levels of oxidative stress that is

increased with ageing [109]. Lipofuscin deposition in AMD is believed to further augment this by inducing an increased level of free radical formation. Similar to brain associated AD pathology, APP processing is likely to be influenced in the retina by exposure to an altered pro-oxidative environment and A $\beta$  can both undergo increased aggregation under such conditions and augment the cellular sensitivity to its neurotoxic effects [108]. AGE receptors (RAGE) bind to A $\beta$  and promote its influx into the CNS across the blood–brain barrier [110]. RAGE are also well expressed in the retina and functionally may play a role analogous to that of the brain by modulating A $\beta$  endocytosis and its flux across the blood retinal barrier. Enhanced RAGE expression has accordingly been observed in the optic disc region of AD subjects [111].

### Involvement of vascular changes

Retinal vascular changes may be early indicators of neurovascular uncoupling in AD [24, 112]. Decrease in retinal blood flow mimicking a situation similar to that associated with A $\beta$  microvascular deposition in brain leading to cerebral hypoperfusion has been suggested [24]. Microvascular A $\beta$  deposition is also reported in the retinas of Tg2576 mouse model of AD [7, 22]. Such amyloid deposition could contribute to local retinal vascular endothelial dysfunction, thereby encompassing dysregulation of vascular parameters in the ambit of A $\beta$  associated retinal disorders [113]. Visual disturbances observed in AD patients may be linked with local narrowing of retinal and choroidal vessels or endothelial dysfunction mediated by A $\beta$  toxicity [3, 24]. Interestingly, a correlation has been observed between retinal microvasculature changes and cognitive performance [114].

A $\beta$  is shown to impair eNOS activity in endothelial cells caused by alterations in intracellular Ca<sup>2+</sup> levels and posttranslational modifications [115]. Transport of A $\beta$  through the blood–brain barrier is affected by eNOS and its inhibition may result in vascular dysfunction, impairment of microcirculation and promote A $\beta$  tissue deposition [116]. Conversely, increased expression of APP and BACE1 as well as increased production of A $\beta$  peptides is detected in the cerebral microvasculature of eNOS-deficient mice [117]. The relevance of these findings in the retina are not yet known although NO is expressed in all retinal cells that plays crucial role to synthesise cGMP and regulate phototransduction [118].

A $\beta$  antibody mediated treatments in AD mice resulted in significant increases in vascular aggregation signifying the involvement of blood vessels in mediating A $\beta$  clearance [119]. This along with the observation that A $\beta$  deposits are observed in choroidal vessels raises concerns that A $\beta$  deposition may also affect the endothelial function, blood

supply and vascular elasticity or permeability in AMD. Interestingly, A $\beta$  induces a marked increase in VEGF production by RPE cells [86] and thus it is likely to have significant implications in retinal neovascularisation in wet AMD. Glaucoma, particularly normal tension glaucoma, is also thought to have a strong vascular component, with reported changes in vascular regulation, retinal vascular pulsation, optic disc haemorrhages and upregulation of hypoxia inducible factor in the retina [6, 120]. High levels of both A $\beta$ 40 and A $\beta$ 42 are reported in the optic disc and region surrounding lamina cribrosa in experimental glaucoma models and human glaucoma ONH [5]. Such A $\beta$  accumulation may cause local disturbances in hemodynamic parameters and also lead to the gradual failure of the elimination of APP cleavage products which can further exacerbate cytotoxic A $\beta$  deposition. Advances in ocular OCT angiography will elucidate the extent of retinal vascular association with each of these diseases and determine potential relationship with A $\beta$  deposition.

### Targeting amyloid $\beta$ in the retina

Deposition of A $\beta$  in retina signifies increased A $\beta$  formation, its aggregation, blunted clearance mechanisms or a combined effect of these deficits. The major question is whether A $\beta$  itself represents one of the pathological causes or is an outcome of the associated retinal pathology. Nevertheless, success in protecting the retina against A $\beta$  accumulation and associated neurotoxicity will stem from greater familiarity with the underlying pathological mechanisms. In this context, efforts to develop drugs for amelioration of A $\beta$ -induced retinal toxicity will benefit from our prior experience in developing brain related therapies. Intervention at early stages before the onset of irreversible neurodegenerative changes in the retina is expected to have better prognosis. In addition to the disease stage, high heterogeneity amongst human populations and varied cohort, sex and age associated responses can lead to differential treatment outcomes. AD management includes using acetylcholinesterase inhibitors for early stages of AD and memantine as the disease progresses [121]. Other drugs such as valproic acid, monoclonal antibodies to A $\beta$  (bapineuzumab, solanezumab, GSK-933776, MABT-5102A),  $\gamma$ -secretase inhibitors (semagacestat, begacestat, etc.) and statins (simvastatin, pitavastatin, atorvastatin, etc.) have been extensively investigated, but the design of clinical trials has not been able to clearly establish statistical significant primary outcomes in AD and no effective drug has been approved by FDA in the last decade [122]. Some of these therapies may find applications in protecting the retina against A $\beta$  toxicity and can potentially be administered locally.

### Can amyloid $\beta$ generation in the retina be scaled down?

Pre-clinical studies indicate that treatments with  $\beta$  and  $\gamma$  secretase inhibitors and with anti- $\beta$  secretase antibodies results in decreased levels of A $\beta$ 40 and A $\beta$ 42 species in the brain [123] (Table 1). In the retina, Guo et al. demonstrated that inhibiting A $\beta$  generation minimises ganglion cell apoptosis in a rodent model of elevated IOP indicating that therapies targeting A $\beta$  generation may have a protective effect in A $\beta$  mediated retinal pathologies [39]. Intravitreal administration of enzyme modulators or neutralising antibodies can lead to decreased A $\beta$  generation in the retina, with minimal systemic side effects. Thiazolidinedione derivative diabetes drugs rosiglitazone and pioglitazone stimulate the nuclear peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) and are potent  $\beta$  secretase inhibitors and may find applications in reducing retinal A $\beta$  build-up. PPAR $\gamma$  receptor activation suppresses APP expression and promotes its ubiquitination and subsequent degradation [124].  $\gamma$  secretase inhibitors such as semagacestat ameliorate A $\beta$  accumulation and additionally cause alterations in the notch signalling pathway [87]. Notch-signalling has

been relatively well characterised in the Muller glial cells in mature retina and blocking notch activity prior to damage is suggested to be protective in various retinal neurons [125]. Non-steroidal anti-inflammatory drugs like ibuprofen and indomethacin selectively affect  $\gamma$  secretase cleavage site and block APP proteolysis leading to reduced A $\beta$  production; however, comprehensive assessment of the safety and efficacy of these drugs in the retinal context needs to be carried out [126]. Similarly, statins in addition to their established cholesterol lowering properties, reduce A $\beta$  production thereby ameliorating A $\beta$  neurotoxicity and might potentially play a role in managing A $\beta$  associated retinal disorders [127].

Experimental and epidemiological data suggest that decline in sex steroid hormone levels is associated with increased AD risk [128]. Interpretation of results from several clinical trials however indicated that hormone replacement therapy (HRT) in women does not improve cognition and one study even showed HRT to have negative effects on cognition [129]. AD subjects depict significant elevation in serum luteinizing hormone (LH) concentrations [130]. Treatments targeting LH can modulate cognitive behaviour in aged APP transgenic mice, and

**Table 1** Table summarising various ways that A $\beta$  can be targeted in retina with examples that are relevant to each strategy

Target	Approach	Examples
A $\beta$ generation	Gamma secretase inhibitors	Semagacestat, begacestat [87]
	$\beta$ Secretase inhibitors	OM99-2, AZD3839, LY2811376, MK8931, CTS21166 [169]
	NSAIDS	Ibuprofen, indomethacin [126]
	Thiazolidinedione derivatives	Rosiglitazone, pioglitazone [124]
	HRT	Leuprolide acetate [131]
A $\beta$ aggregation	Natural compounds/extracts	Green tea, <i>Ginkgo biloba</i> extract, curcumin, inositol [132, 170]
	Small molecule compounds	Nitrophenols, curcumin [132, 133, 138]
	Metal chelators	Clioquinol, PBT2 [139, 140]
	Monoclonal antibody therapy	Bapineuzumab, solanezumab, GSK-933776, MABT-5102A [152, 153]
A $\beta$ clearance	Peptidic and non-peptidic compounds	RS-0406, SEN1269 [141]
	Metal chelators	PBT2 [140]
	Antibody treatment	Bapineuzumab, solanezumab, GSK-933776, MABT-5102A, anti-A $\beta$ (2H6-D) [11, 142, 152, 153]
	Immunotherapy	AN-1792, CAD-106, Lu AF20513, ACC-001, V-950, ACI-24, UB-311 [148, 149]
	RXR agonists	Bexarotene [154–156]
	RAGE-binding competitors	TTP488, FPS-ZM1 [157]
Neuroprotective strategies	Neurotrophins	BDNF gene therapy [166]
	Soluble Nogo-receptors	Soluble Nogo-66 receptor [167]
	Acetylcholine esterase inhibitors	Donepezil, rivastigmine, galantamine [162, 163]
	Voltage-dependent NMDA receptor agonists	Memantine [158–160]
	Anti-histamine drugs	Latrepirdine [161]
	Group IV PLA2 targets	Quinacrine, CHEC-9 [164]

also reduce A $\beta$  deposition. Gonadotropin-releasing hormone (GRH) agonist leuprolide, suppresses LH by down-regulating pituitary GRH receptors and is suggested to be an effective treatment for AD [131]. LH receptors are expressed in the neural retina and their pharmacological modulation may help prevent A $\beta$  accumulation.

Additionally, several plant extracts and natural compounds have been demonstrated to promote non-amyloidogenic APP processing and reduce toxic A $\beta$  generation and prevent its aggregation by multiple and complex mechanisms. Epigallocatechin-3-gallate for example, is present in green tea, which reduced amyloidogenesis in animal models of AD [132]. Antioxidants such as lipoic acid, ginkgo biloba extract, *n*-acetyl cysteine, flavonoid derivatives, vitamin E, curcumin, ubiquinone are also demonstrated to have protective effects in AD and reduce amyloidogenesis [133]. Interestingly some of these supplements have been suggested to exhibit protective effects in retina in AMD and glaucomatous conditions [134]. Dietary antioxidative supplements containing vitamins C, E, Zn and  $\beta$  carotene significantly reduced the AMD progression. Similarly, over-expression of antioxidant enzymes thioredoxins 1/2 showed a protective effect on RGCs in glaucoma while SOD1 deficient mice exhibited increased RGC loss [135, 136].

### Can neurotoxic effects of A $\beta$ be silenced by preventing its aggregation?

Amyloid  $\beta$  monomers tend to self-aggregate into various assemblies such as monomers, oligomers, protofibrils, fibrils and amyloid plaques. It is suggested that A $\beta$  monomers can be neuroprotective, though conversely the aggregation of A $\beta$  may contribute to AD pathology (Fig. 3). Soluble A $\beta$  oligomers are also reported as being more harmful than monomeric or fibrillar forms and promote synaptic loss [137]. Several natural and synthetic molecules have been shown to prevent A $\beta$  aggregation [132, 133]. Treatment with curcumin and nitrophenol for example, disrupted existing amyloid plaques and in some studies even partially restored the distorted neurite network [133]. Curcumin is shown to bind A $\beta$  aggregates in the retina and protect the retinal neurons against excitotoxicity and oxidative damage [20, 138].

Clioquinol is a specific Cu<sup>2+</sup>, Zn<sup>2+</sup> chelator and markedly inhibited cerebral A $\beta$  aggregation in animal models of AD as well as in the patients in phase 2 trials. It readily penetrates the BBB due to its hydrophobic properties and may find therapeutically effective role in preventing A $\beta$  deposition in the retina; however, careful investigations need to be carried out in the light of subacute myelo-optic neuropathy reports associated with its administration [139]. PBT2 also binds Cu<sup>2+</sup> and Zn<sup>2+</sup> ions and has even higher

BBB permeability than clioquinol and its treatment reduced levels of soluble and insoluble A $\beta$ . In the phase 2 trials, PBT2 was effective in reducing CSF A $\beta$ 42 levels in AD subjects [140]. PBT2 may, thus, have more bioavailability and effectively reduce A $\beta$  load in vitreous and retina. Similarly, peptidic and non-peptidic compounds (RS-0406, SEN1269) that bind to A $\beta$  and block its self-aggregation seem to work well in culture conditions [141], and given the ease of delivery and relatively lower A $\beta$  load, retina will be a good model system to investigate their efficacy in vivo. Further, several antibodies targeting A $\beta$  conformation have been developed to target its aggregation and tested positively in animal models of AD [142, 143] and some of these are currently undergoing clinical trials (Gammagard-Baxter, Gantenerumab-Roche, AAB-002-Elan) [144] (Table 1).

### Will enhanced amyloid $\beta$ clearance from retina be helpful?

Promoting A $\beta$  clearance from retina has the potential to slow down or rescue the structural and functional defects associated with A $\beta$  linked retinal disorders. Several pharmacological and immunotherapies have been tested while others are under development and have illustrated varying degrees of success in modulating A $\beta$  levels in preclinical and clinical trials in AD [39, 143, 145, 146]. A $\beta$ 42 vaccination in young Tg2576 mice protected against A $\beta$  deposition in brain while in aged animals it was not as efficacious in eroding already formed A $\beta$  assemblies. A $\beta$ 42 immunisation also resulted in cognitive benefits and reduction in soluble A $\beta$  in aged 3xTg mice suggesting that treatment may be more effective before the A $\beta$  deposits are formed [143, 146]. However, A $\beta$ 42 immunisation approach (AN-1792) met with serious side effects such as meningoencephalitis possibly attributed to increased influx of T lymphocytes in brain and this needs to be considered when developing analogous therapies for the retina [147]. Various vaccines such as Lu AF20513, CAD-106, ACC-001, V-950, ACI-24, UB-311 have been developed with the aim to minimise the cellular and maximise the humoral immune responses [145, 148]. Phase 2 and 3 longitudinal trials in AD subjects are being conducted using next generation vaccines (CAD106) comprising much smaller A $\beta$  sequences to minimise the immune response [149]. Nucleic acid-A $\beta$  immunotherapy is another approach which is being evaluated in mice models of AD for non-inflammatory immune responses to target A $\beta$  [150].

A $\beta$  antibody therapies which target amino or carboxy terminal, middle part, different conformational states and aggregated forms of A $\beta$  are under investigation. Antibody studies indicate reduction in amyloid load, plaque formation and improvement of cognitive function in mouse

models of AD [151]. Humanised monoclonal antibodies (bapineuzumab, ponezumab and solanezumab) directed against different regions of the A $\beta$  peptide have been tested in the clinic (Table 1). Although initial trials showed various cognitive benefits especially in ApoE/E4 non-carriers, overall results from larger phase 3 trials failed to achieve expected end-points and were also associated with vasogenic edema and associated MRI signal abnormalities and microhemorrhages [152, 153]. Modifications in antibody design may help ameliorate some of these side effects and phase 3 trials on such A $\beta$  antibodies including aducanumab, gantenerumab, crenezumab, BAN2401, etc. are currently in progress in both MCI and AD subjects [121, 144, 149]. The deglycosylated form of an anti-A $\beta$ 40 (2H6-D) treatment in Tg2576 mice quite interestingly exhibited a significantly lower incidence of haemorrhagic and inflammatory brain lesions [142]. Setbacks associated with immunotherapies do not necessarily indicate that A $\beta$  is the wrong target as these shortcomings could also arise from low passage of antibodies across the BBB and consequent inability to achieve required tissue levels. Besides, immunotherapeutic approaches could be most effective in the initial stages of the disease and effects may not be evident if the intervention is too late. Anti-A $\beta$ 40/42 mAb therapy targeting c-terminal of A $\beta$  peptides resulted in decreased AMD-like pathology, improved visual acuity and protected against RPE structural changes and retinal functional deficits in a dose-dependent manner [11]. Antibody therapy also resulted in reduced RGC apoptosis in animal model of glaucoma [39]. Reduction in A $\beta$  deposition and functional improvements indicated that approaches to enhance A $\beta$  clearance represent a viable therapeutic option that needs to be investigated in the retinal context and refined to enhance efficacy and minimise side effects [20]. Efficacy of A $\beta$  antibody therapies has not been evaluated for the retina in animal models of AD but represents a valid option to target soluble and aggregated A $\beta$  through intravitreal access, which will also help minimise the systemic side effects.

A $\beta$  levels can also potentially be modulated by stimulating increased uptake by glial cells. In this regard, activation of retinoid x-receptors (RXRs) in brain using RXR agonist bexarotene was explored to promote glial uptake of soluble A $\beta$  to enhance clearance [154]. Independent follow-up studies, however, failed to confirm the protective effects of the drug or establish any beneficial effects on memory and cognition in animal models, with only few groups being able to confirm lowering of soluble A $\beta$  levels [155, 156]. Given that RXRs are well expressed in the retina, alternative strategies to target them can have differential tissue specific effects on A $\beta$  levels in the retina and help unravel the pathophysiological cross talk between

RXR and A $\beta$ . The soluble form of RAGE (sRAGE) has also been shown to compete for A $\beta$  binding with the membrane-linked RAGE, thus promoting removal of circulating A $\beta$  and its antagonists are being tested in clinical trials [157]. Monitoring in vivo retinal changes will provide significant inputs about potential applications of these treatments in A $\beta$  linked ocular pathologies.

### **Do we have efficient neuroprotective therapies to buffer detrimental effects of A $\beta$ in retina?**

No medications or treatment strategies are yet credibly established in the clinic to specifically increase cerebral or retinal tolerance to neurotoxic effects of A $\beta$  although several preclinical and clinical studies have suggested neuroprotective effects of various therapies in AD. Memantine, for example, reduces neurotransmission rates and is approved for dementia management and promotes reduction in A $\beta$  toxicity in AD and vascular dementia; prevents tau hyperphosphorylation; decreases microglial activation and promotes release of neurotrophic factors from astroglia [158]. This drug also protected RGCs in mice models of inner retinal damage [159], but phase 3 glaucoma trial did not achieve primary endpoints [160]. Nevertheless, its role in decreasing A $\beta$  or imparting protection against its toxicity in the retina was not considered in these studies. With the advent of newer in vivo A $\beta$  and apoptotic cell imaging technologies, potential protective effects of memantine on A $\beta$  toxicity in retina could be evaluated in further detail in AMD, AD and glaucoma. Latrepirdine, which is an antihistamine drug, inhibited the formation of mitochondrial permeability transition pore and protected neuronal mitochondria from A $\beta$  mediated toxicity [161]. Latrepirdine may, therefore, find applications in protecting retinal mitochondrial dysregulation in AMD and glaucoma against A $\beta$  effects. Acetylcholinesterase inhibitors such as donepezil, rivastigmine, galantamine are also widely used in AD management [162]. These are also suggested to boost retinal vasoprotection and enhance ocular blood flow in experimental models of glaucoma [163]. Detailed investigations on whether acetylcholinesterase inhibitor mediated retinal protection is facilitated by modulation of A $\beta$  neurotoxic effects will provide mechanistic insights and unravel new lines of disease management. Another potential approach could be pharmacologically or genetically targeting group IV phospholipase A2 which is well expressed in the retinal tissues to reduce toxic effects of A $\beta$  deposition as down-regulation of this enzyme has previously been shown to exhibit protective effects in animal model of AD [164] (Table 1). Similarly, T cell mediated microglial activation was associated with reduced A $\beta$  deposition and enhanced

neurogenesis. Administration of T-cell modulating antigens exerted protective effects in AMD by reducing the drusen deposition [6, 165].

Gene therapy approaches to modulate neurotrophins such as BDNF have been demonstrated as beneficial in animal model of AD [166]. BDNF plays a critical role in the protection of RGCs in glaucoma and its impairment is associated with increased retinal A $\beta$  [5]. BDNF gene therapy can rescue the retina, particularly RGCs, but the protective effects are transient and repeated administration or higher doses do not generally prolong the effects. Therefore, upstream regulators or downstream effectors of BDNF/TrkB signalling may serve as better therapeutic targets to improve BDNF/TrkB signalling [5, 66]. Both APP and A $\beta$  bind to the Nogo receptor and its downregulation promoted increased levels of A $\beta$  in the brain and plaque formation in animal models of AD. Treatment with soluble Nogo receptor was shown to reduce A $\beta$  aggregation [167] and interestingly, soluble Nogo-66 receptor also prevented synaptic impairment and rescued RGC loss in chronic glaucoma, while Nogo upregulation induced RGC death [168]. Nogo signalling thus can be another potential therapeutic target for gene therapy or pharmacological intervention directed towards modulating A $\beta$  effects.

### Limitations and future directions

This review has highlighted the involvement of A $\beta$  accumulation in retina in AD, AMD and glaucoma and its complex pathophysiological effects on the eye. Various strategies to manage A $\beta$  accumulation and ameliorate its effects are discussed; however, predominantly non-mendelian genetic basis of these diseases designate that a single cure may not be easily achievable in the near future. Accumulating evidence from human and animal studies suggests that these three are distinct disease conditions but with overlapping A $\beta$  related neurodegenerative processes. Analysing A $\beta$  characteristics including fragment size, oligomerisation and anatomical distribution within the retina holds “reasonable promise” to provide unique retinal signatures and differentiate between various A $\beta$  associated disease conditions. For instance, AMD-associated A $\beta$  deposition is more restricted to RPE and likely to be identified as complex with drusen [71]. In glaucoma, expression of A $\beta$  could be found in the inner retina [5, 39] along with ON excavation. Retinal A $\beta$  deposition in AD is geographically more wide spread and again associated with degenerative changes in the inner retina and ON [1]. Successful identification of differences and similarities will drive drug development and mechanism based pathophysiological research. Imaging patterns of A $\beta$  deposition in the eye could be of diagnostic and prognostic value when

considered with other ocular or biochemical markers and play imperative role in disease monitoring. AD is mainly a brain disease and clinical evaluation of retinal involvement promises to help in AD diagnosis and prognosis. Development of newer A $\beta$  imaging technologies will greatly accelerate research into mechanism based therapies for these neurodegenerative disorders. Longitudinal studies in larger cohorts and rescue experiments will support the hypothesis that A $\beta$  deposition does exert neurotoxic effects on the retina. Concluding, the significance of A $\beta$  deposition in mechanistic understanding of retinal pathology, disease diagnosis, prognosis or as a treatment target is highly relevant considering its known neurotoxic effects and the high incidence of these diseases in ageing populations.

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