Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder, the most common form of dementia. AD is characterised by amyloid-ß (Aß) plaques and neurofibrillary tangles (NFT) in the brain, in association with neuronal loss and synaptic failure, causing cognitive deficits. Accurate and early diagnosis is currently unavailable in lifespan, hampering early intervention of potential new treatments. Visual deficits have been well documented in AD patients, and the pathological changes identified in the brain are also believed to be found in the retina, an integral part of the central nervous system. Retinal changes can be detected by real-time non-invasive imaging, due to the transparent nature of the ocular media, potentially allowing an earlier diagnosis as well as monitoring disease progression and treatment outcome. Animal models are essential for AD research, and this review has a focus on retinal changes in various transgenic AD mouse models with retinal imaging and immunohistochemical analysis as well as therapeutic effects in those models. We also discuss the limitations of transgenic AD models in clinical translations.

INTRODUCTION

Alzheimer's disease (AD), the most common cause of dementia, represents a huge global challenge in health care for a rapidly ageing population. An estimated 47 million people were living with dementia worldwide in 2015, a number that is expected to triple by 2050 [1]. The overwhelming health problems associated with AD are largely due to the absence of an early and accurate diagnosis, thwarting any opportunity for disease-modifying treatment or cure. Although a century has passed since the first case of AD was identified [2], a definitive diagnosis of AD is still made via an autopsy, by revealing two cerebral hallmarks: extracellular amyloid-ß (Aß) plaques and intracellular neurofibrillary tangles (NFT). However, recent clinical evidence suggests that a combination of cerebrospinal fluid (CSF) biomarkers with neuroimaging techniques to visualize cerebral Aß deposition would make it possible to better identify individuals at the greatest risk of developing AD [3]. Nonetheless, routine clinical practice of these approaches remains prohibitive due to their cost and invasive nature. There is therefore a dire need to search for non-invasive, readily accessible, and inexpensive biomarkers for the early detection of AD, enabling early therapeutic interventions to be implemented.

Ocular biomarkers have been explored since discoveries that the eye, particularly the retina, is also affected in AD. The retina, as an integral part of the central nervous system (CNS), shares the same embryological origin as the brain. Furthermore, the eye shares anatomical and physiological similarities with the brain, such as between the blood-retinal barrier and blood-brain barrier, and between the aqueous humour and CSF. Due to the optically transparent nature of the eye, non-invasive real-time cost-effective imaging of the retina is clinically feasible, potentially allowing earlier diagnosis as well as monitoring of disease progression and treatment outcome.

Since the first evidence of retinal degeneration in human AD was reported by Hinton three decades ago [4], AD-associated retinal changes have been identified, including neuronal degeneration and loss, thinning of the retinal nerve fibre layer (RNFL), optic disc cupping, and visual function impairment. Most significantly, retinal Aß plaques were detected in post-mortem retina from AD patients but not age-matched non-AD individuals [5]. Thus, the concept of the retina as a window into the disease of the brain has received increasing attention and interest in recent years [6–9]. While research using living human eyes is inherently problematic, much progress in searching for retinal biomarkers has been made using transgenic AD (Tg-AD) mice. This review will cover retinal changes that have been detected in various Tg-AD mouse models with retinal immunohistochemical analysis and in vivo imaging as well as therapeutic effects in those models.

THE RETINA AND BRAIN

Having extended from the neural tube during embryonic development, the retina is an integral part of the CNS, and importantly, is an area that has evolved to optimise the exposure of the central nervous system to light. By observing physiological conditions in the retina, we are provided with a unique and non-invasive window into potential pathologies of the brain.

The retina is formed by three layers of neurons, with the most exterior layer being the outer nuclear layer (ONL), consisting of photoreceptors (cones and rods), responsible for the transduction of light energy to action potentials. This is followed interiorly by the inner nuclear layer (INL), comprising of amacrine cells (AC), bipolar cells (BC), and horizontal cells (HC), which are involved in the organisation of visual information received from the outer nuclear layer. The final and most inner layer of the retina is the ganglion cell layer (GCL), where retinal ganglion cells (RGCs) receive input from the outer and inner nuclear layers and project posteriorly with their axons forming the optic nerve (ON). The optic nerve finally carries the visual signals into the brain through the visual pathway, via the lateral geniculate nucleus (LGN) and superior colliculus (SC), before reaching the visual cortex (VC). See

Figure 1 for an illustration of the retina and brain. The visual pathway is affected at any level of neurons, from the retina to the visual cortex, in Alzheimer's disease [6].

Along with the visual pathway, there is another anatomical and functional distinguished pathway named the circadian pathway. In this pathway, a small subset of RGCs - melanopsin RGC (mRGCs), as photoreceptors carry photic information, through the retinohypothalamic tract (RHT), to the hypothalamic suprachiasmatic nucleus (SCN) in the brain [10]. The mRGCs play a critical role in the circadian rhythm which has been reported to be disrupted in Alzheimer's disease [11].

TRANSGENIC MOUSE MODELS OF AD (Tg-AD)

Genetic modification is established on the three gene mutations linked to familial AD (FAD), which are amyloid precursor protein (APP), presenilin-1 (PS1), and presenilin-2 (PS2), inherited in an autosomal dominant manner. Although accounting for less than 1% of AD cases, FAD provides a substantial amount of information in creating AD models, and basic AD research on those models has significantly advanced our knowledge of AD. AD transgenic models have become invaluable tools to study ocular biomarkers for early diagnosis and evaluate potential treatment in a whole organism.

Most transgenic models of AD are made in mice, due to firstly, having a similar CNS structure to humans, and secondly, being relatively easy and inexpensive to produce. Currently, over 205 different transgenic AD models are available for research (http://www.alzforum.org/res/com/tra), and they are categorised into single, double, triple, and x5 transgenic genotypes (based on the number of genes that have been modified).

The most common strategy to generate an AD model is overexpression of FAD-associated mutant APP, and almost half of APP transgenic mice overexpress the Swedish double mutation (K670N/M671L) of APP (APP_{swe}), such as the Tg2576 mice, the most widely used model with this mutation. Tg2576 mice develop an age-dependent cognitive impairment and Aß plaques in the brain, along with other pathological changes [12, 13]. Tg2576 mice also produce oligomeric Aß, which seems to be more toxic than fibrillary or aggregated Aß in plaques. However, these mice fail to show any significant neuronal loss in the brain [14]. In addition to the Swedish mutation, the APP transgene contains other mutations that facilitate aggregation and generation of the more toxic Aß42 peptide, such as E693G (Arctic), E693K (Dutch), V717F (Indiana), or V717I (London). In general, these transgenic mice develop much more aggressive Aß pathologies than Swedish APP mice [15].

Presenilins (PSENs) are the catalytic core of the γ-secretase complex which is involved in APP processing. Mutations in presenilins are associated with reduced γ-secretase cleavage of APP, resulting in increased Aß generation and deposition in FAD [16]. Mice expressing mutant PSEN1 or PSEN2 have been generated accordingly. Unlike APP models, presenilin transgenic mice do not develop plaque pathology in the brain. This is explained by the fact that the Aß sequence differs between mice and humans, reducing the tendency of Aß to aggregate in mice [17]. To overcome this issue, double transgenic mice have been created by introducing human APP into presenilin transgenic mice; these mice exhibit robust Aß42 generation and Aß plaque formation at much earlier ages than APP mice [18].

In addition to Aß, tau pathology is also associated with AD. However, in contrast to APP, no genetic mutation of tau has been identified in FAD, indicating that tau pathology in AD may be downstream of APP/Aß pathology. To generate tau transgenic models, FTDP-17 tau mutations have been introduced into mice. The first of these models was generated using tau (P301L), the most common mutation in FTDP-17, under the control of mouse prion promoter [19]. Subsequently generated models harbouring various FTDP-17-associated tau mutations exhibit similar neuropathology to AD, including robust NFT-like tau pathology, neuronal and synaptic loss, and cognitive impairments in an age-dependent manner [20–23]. This also indicates that NFT-like neuropathology can form in the absence

of amyloid pathology. However, it is also evidenced that the formation of NFT-like neuropathology in mutant tau mice is significantly accelerated following the addition of Aß fibrils [24]. In this regard, it is crucial to generate a model that develops both Aß and Tau neuropathology.

A triple transgenic mouse model (3xTg-AD) was therefore created in a PS1M146V knock-in mouse, by harbouring three disease mutant genes, i.e. two Swedish mutations (K670N/M671L) on APP695 and one FTDP-17 tau (P301L) mutation [25]. The 3xTg-AD mouse develops age-dependent Aß and tau pathologies, along with other AD-related pathologies, including astrogliosis, activated microglia, loss of synapses, and neurodegeneration [26–28].

5xFAD mice express human APP and PSEN1 transgenes with a total of five AD-linked mutations, which are the Swedish (K670N/M671L), Florida (I716V), and London (V717I) mutations in APP, and the M146L and L286V mutations in PSEN1. The 5xFAD mice rapidly develop severe amyloid pathology with a high level of intraneuronal Aß42 occurring as early as 1.5 months of age [29]. Extracellular Aß plaques begin at 2 months and increase rapidly with age, with amyloid pathology more severe in females than males [30]. Neuron loss occurs in multiple brain regions [29], and mice display a range of cognitive and motor deficits [31].

For more comprehensive reviews on transgenic AD models refer to Kitazawa M et al. [15], Duyckaerts C et al. [32], Mullane K et al. [33], Drummond E et al. [34], and Myers A et al. [35].

RETINAL HISTOLOGICAL CHANGES IN Tg-AD

Retinal changes in transgenic AD mice are mostly studied by immunohistochemistry. A summary of retinal histological changes in various Tg-AD mice is presented in Table 1.

Amyloid-ß deposition

Several studies have reported amyloid-ß retinal immunoreactivity in Tg-AD mice, including Tg2576 (single), $APP_{swe}/PS1_{\Delta E9}$ (double), and the 3xTg-AD (triple) transgenic mice. A few studies present conclusive evidence of amyloid plaque-like deposits in the retina by using a battery of amyloid-ß specific antibodies and special stains [5, 36, 37]. However, there are also a growing number of reports which indicate an absence of amyloid deposits in the retina [38][39][40]. We will discuss these findings, note possible disparities, and consider options and implications of using different transgenic models for AD-related retinal studies.

The Tg2576 mouse is a commonly used single transgenic model in AD research. Aß plaques in the brain typically start at 9 months, followed by an age-dependent increase with significant plaques seen at 11-13 months of age [41]. At the age of 14 months, Aß plaque-like deposits are present in the retina, including the RGC layer, the outer nuclear layer, and the photoreceptor outer segments as well as the optic nerve, identified by multiple anti-Aß antibodies and Congo-red [36]. However, not all studies agree - in one study, for example, retinal extracellular Aß plaques have not been observed at a similar age of Tg2576 mice, though cytoplasmic Aß is identified in the RGC layer and inner nuclear layer [38]. In another transgenic model (TgCRND8), where brain Aβ deposition is seen at 3 months, positive amyloid aggregates are detected at 4 months in the RGC layer, hence postulating the reason for RGC loss and RNFL thinning reported in this model [42].

The double $APP_{swe}/PS1_{\Delta E9}$ mice have been the most commonly used model for AD-related retinal histopathological studies. Compared to Tg2576, these mice exhibit earlier and larger plaque deposition in the brain starting at an early age of 6 months [43]. In the retina, the onset of Aß deposition may differ with strains. For example, Ning et al. compared the two double transgenic strains of APP/PS1 (Tg2576xTg1 and $APP_{swe}/PS1_{\Delta E9}$) and found that the latter exhibits Aß immunoreaction in the nerve fibre layer at 10.5 months old, while retinal Aß deposits have not been detected in the former at earlier

(7.8 months), but later (27 months) ages [44]. Aβ deposits are also found in retinal whole mounts [45] and in the inner and outer plexiform layers of retinal cross-sections in the $APP_{swe}/PS1_{\Delta E9}$ mice, with female mice appearing to have earlier deposition than males [37]. Studies have shown that retinal Aß appears to occur in older Tg-AD mice when brain amyloid pathology is already established [37, 44, 45]. However, a recent study on the same model described retinal Aß deposits as early as 2.5 months of age, preceding brain pathology, in both retinal whole mounts and cross-sections [5]. Nevertheless, conflicting findings exist where early Aß deposition in the retina cannot be identified in $APP_{swe}/PS1_{\Delta E9}$ mice [39]. The absence of Aβ plaques in the retina has been explained as being due to non-amyloidogenic processing of APP taking place in the retina as opposed to the brain in this model [46].

Triple Tg-AD mice have also been studied for retinal Aß deposition. Compared to single and double Tg-AD models, Aß deposition has been observed much earlier (as early as 5 weeks of age) in the inner and outer retinal layers, before the establishment of amyloid deposits in the brain [47]. Positive Aß immunoreactivity has also been seen in retinal whole mounts in older (9-12 months old) 3xTg mice [48]. Nonetheless, the absence of amyloid deposits in the retina has been reported (unpublished data) in all three forms of transgenic mice including aged 3xTg mice [40].

Retinal amyloid pathology has also been assessed in 5xFAD mice. Intracellular Aß accumulation has been identified in the retinal pigment epithelium (RPE) in aged 5xFAD mice, along with large vacuoles, hypopigmentation in the RPE layer, and thickened Bruch membrane and drusen-like deposit between RPE and Bruch's membrane – the features of dry AMD (age-related macular degeneration) [49, 50]. Intracellular accumulation of Aß in the RPE compromises their integrity as tight junctions, causing the breakdown of the outer blood-retinal barrier in 5xFAD mice [49]. Accelerated Aß deposition in the retina of 5xFAD mice is associated with progressive inflammatory pathology which is further enhanced by aluminum supplements [51]. Among AD mouse models, 5xFAD mice have shown the highest retinal abundance of $Aß_{42}$ and the highest deficits in complement components using ELISA [52]. There is no study yet to look into histological changes of Aß pathology in the neural retina.

A number of variables may contribute to disparities between the studies. Differences in immunohistochemistry protocols and choice of Aβ antibodies could partly explain the variability seen in the same animal model when studied by different groups [53]. In addition to the absence of Aβ deposition shown in wild type controls, it would be good practice to validate the positive staining by using isotype controls to rule out false positives [54]. An important variable in plaque deposition is gender-specific differences, with published reports indicating gender-specific pathological differences in Tg2576 and APP/PS1ΔE9 mice [55, 56]. The Jackson laboratory website states a communication, made by the source laboratory of 3xTg-AD, that the male transgenic mice exhibited discrepancies in phenotypic traits from what was originally described (https://www.jax.org/strain/004807). Additionally, a proportion of the Tg2576 mice from the B6:SJL genetic background are predicted to have the rd1 retinal degenerative mutation, which directly affects aspects of behavioural testing [57], and would not be an ideal choice for histological studies on retinal degeneration in Alzheimer's disease.

Hyper-phosphorylated tau

Alongside senile plaques, another important neuropathological hallmark of AD is the presence of neurofibrillary tangles in the brain, caused by multi-site phosphorylation of microtubule-associated protein tau. Research into *in vivo* imaging of abnormal tau in the brain, using PET tracer techniques, has been gaining attention in recent years [58]. Tau pathology has recently been implicated in wake-promoting neuron degeneration in early AD, associated with sleep-wake disturbances in the circadian pathway [59]. On the therapeutic side, the focus is now shifting towards drugs that target tau, following the failure of some drugs targeting Aß in clinical trials [60]. In light of this shift, we will discuss studies that have looked at retinal tau changes in Tg-AD mice.

The single transgenic Tg2576 and the double transgenic APP/PS1 mice do not carry a tau mutation, however, based on the hypothesis that Aß deposition and tau pathology are linked, some studies looked at these mice and found that immunoreactivity for hyper-phosphorylated tau (pTau) also existed in the brain [61, 62]. pTau is also found in the retina of these mice, and the localisation of pTau corresponds to Aß deposition in Tg2576 [36]. In $APP_{swe}/PS1_{\Delta E9}$ mice, both retinal pTau and Aß deposition appeared to be reduced following bone marrow transplantation [63]. Additionally, upregulation of calpain may contribute to tau hyperphosphorylation in the retina alongside Aβ deposition in APP/PS1 mice [64].

Unlike the mutations in APP and PS1 genes, which are directly linked to familial Alzheimer's disease, causal tau mutations in AD have not been established. Hence, tau transgenic models that incorporate the MAPT mutation linked to frontotemporal dementia have been used to recreate tau pathology, either in single transgenic models or multi-transgenic models [65]. Gasparini et al. studied tau-driven neurodegeneration in the retina in P301S tau transgenic mice and found accumulated p-Tau in the nerve fibre layer and RGC layer [66]. Positive staining for p-Tau was also seen in retinal whole mounts in the P301S mice [40]. In 3xTg-AD mice, which carry a tau (P301L) mutation, retinal p-Tau was observed from 5 weeks onwards in the RGC layer, while increasing p-Tau volume was seen in other retinal layers in older animals [47]. Age-dependent increases in retinal endogenous tau were also observed in the 3xTg-AD mice and the accumulated tau was postulated to disrupt anterograde axonal transport [67]. Interestingly, hTau mice, which express only human tau isoforms and develop neurofibrillary tangles in the brain, were not found to exhibit impaired function or survival of retinal cells [68].

The above studies are promising in terms of detecting tau tangles in the retina and their possible effect on AD. Keeping in mind the variables of gender and age, it would be valuable to establish the timescale of tau tangle development in the brain for commonly used AD models before studying the correlation between the brain and retina. This would help towards providing a solid basis for research into the development of imaging techniques for visualization of tangles in the retina, and thus an early diagnosis of Alzheimer's disease.

Glial Changes

Over the last decade, there has been increasing interest in understanding the role of neuron-glia interactions and the pathophysiology of the glial cells - microglia and astrocytes - in neurodegenerative diseases, such as Alzheimer's disease. Both types of glial cells are crucial for immune surveillance and homeostasis of the CNS. They are known to have neuroprotective as well as neuroinflammatory activities in the brain and are increasingly being seen as therapeutic targets for neurodegeneration [69]. The retina has three distinct glial cell types: Müller cells, astrocytes, and microglia. Müller cells and astrocytes are both macroglia, which undergo a phenomenon called reactive gliosis when there is a departure from normal physiological conditions in the retina, such as neurodegeneration, neuroinflammation, or ischemic damage. Several markers, such as glial fibrillary acidic protein (GFAP), are upregulated during this process and can be used for immunohistochemical identification [70].

The microglia play a role in immune surveillance of the retina and are activated by changes in the retinal microenvironment [70]. In an AD brain, it is hypothesized that protein aggregation, such as amyloid plaques, results in microglial activation, causing microglia to surround the plaques to clear them. On the other hand, chronic microglial activation can also exacerbate AD pathology. Microglial activation in the brain has been reported to be different between AD transgenic models and humans, which is largely attributed to the rapid accumulation of Aß plaques in AD models contrasting with the slower chronic accumulation of plaques in humans [71].

The glial activity has been studied in the retina of Tg-AD mice. Increased astrocyte reactive gliosis is seen in retinal whole-mount from a single Tg-AD model (Tg-SWDI). Increased GFAP immunoreaction was also observed in the nerve fibre layer in an age-dependent manner, relative to wild type controls [72]. However, while no evidence of increased GFAP immunoreactivity was found in $APP_{swe}/PS1_{\Delta E9}$ mice [37], microglial activation, identified by the F4/80 microglial marker, appeared to increase with age in the retina in double transgenic mice, along with the presence of Aβ immunoreactivity [32]. Increased morphological changes in retinal microglia were also noted in the double transgenic mice [37]. The relationship between Aß and glial activation is seen in Müller cells of 3xTg-AD mice, where Aß deposition is surrounded by glial processes, which supports the hypothesis that Aß associated neuronal changes are accompanied by glial activation [48]. Furthermore, in 3xTg-AD, increased retinal GFAP immunoreaction and changes in microglia phenotype appeared to occur at an early age, while increased microglial density was seen in older animals [47].

Neuronal loss

Neuronal cell loss is a crucial pathological feature of Alzheimer's disease, where the accumulation of amyloid plaques and neurofibrillary tangles results in disruption of healthy neuronal functions, leading to cell death. Although transgenic AD mice are successfully modelled in the accumulation of plaques and tangles in the brain, they have been less successful in modelling the neuronal loss seen in human post-mortem brains [73]. Because of this, it would be interesting to study whether these animal models provide insight into neuronal loss in the retina where pathological features such as amyloid deposition, tau tangles, and gliosis have been seen.

Some of the above-mentioned histological studies on the Tg-AD retina have also studied neuronal cell loss using techniques such as TUNEL for apoptosis, or standard but quantitative haematoxylin and eosin staining of the retina. For example, Oliveira-Souza et al. reported that TgSw-DI retinas had fewer cells in all layers compared to WT [72]. Similarly, other studies showed a reduction of RGCs in 7.8-month-old Tg2576xTg1 and 10.5-month-old $APP_{swe}/PS1_{\Delta E9}$ transgenic mice, with this reduction being shown to be age-dependent in the former model [44]. In the same animal model ($APP_{swe}/PS1_{\Delta E9}$), the RGCL had a lower cell density and the inner plexiform layer was thinner compared to control animals [45]. In another study, Tg2576 mice exhibited a decrease in retinal thickness, indicating a neuronal or photoreceptor cell loss [36, 74]. In 3xTg-AD mice, RGC apoptosis was associated with anterograde axonal transport impairment caused by tau accumulation [67]. RGC apoptosis was also reported in another Tg-AD model, TgCRND8, along with GCL thinning detected by optical coherence tomography (OCT) [42].

Although many studies have shown a neuronal loss in the retina, this remains controversial, as some studies have reported opposite results. Perez et al. did not find retinal thickness reduction in 12-16-month-old $APP_{swe}/PS1_{\Delta E9}$ transgenic mice, suggesting no retinal loss occurred [37]. Likewise, human P301S tau transgenic mice did not show a lower RGC count compared to wild-type C57/Bl6 mice [66]. However, these studies need to be considered with caution, as retinal thickness reduction can only be detected after a significant neuronal loss occurs, and hence is not sufficiently sensitive to be the sole endpoint of a study. Furthermore, tau transgenic AD mice might not show RGC loss as they do not express Aß, which could be necessary to induce RGC apoptosis [75, 76].

Encouragingly, most of these findings correlate with what is reported in AD patients. Since the first evidence of retinal degeneration was identified by post-mortem analysis in AD patients [4], many studies have reported RGC loss along with retinal nerve fibre layer thinning, and other neuronal loss and retinal thinning in the INL and ONL in AD patients [64–67]. A reduction of 25% of RGC number in the foveal and parafoveal area [79] and a reduction of up to 59% in other areas [81] was reported.

Also, an increased number of astrocytes in the retina, with a significantly higher ratio of astrocytes to neurons, was noted in AD patients [79].

All in all, these encouraging results underscore the importance of the eye in the pathophysiological processes of AD and might allow an easier diagnosis in the near future.

RETINAL *IN VIVO* CHANGES IN Tg-AD

Retinal changes have been studied *in vivo* in Tg-AD mice, including retinal structural and functional impairments, detected by *in vivo* imaging and electroretinography (ERG), respectively. A summary of retinal changes is presented in Table 2.

In vivo imaging

Retinal real-time imaging to search for AD biomarkers is emerging in recent years using Tg-AD mice.

Aß plaques were reported to be identified *in vivo* in the retina of Tg-AD mice. After verifying that curcumin could specifically label Aß plaques *ex vivo* in the retina of Tg-AD mice ($APP_{swe}/PS1_{\Delta E9}$), *in vivo* labelling of Aß plaques was tested by systemic administration of curcumin [5]. Curcumin-labelled plaques were histologically detected in both the retina and brain in the Tg-AD mice but not in WT controls. Curcumin-labelled retinal plaques were also reportedly visualized by *in vivo* imaging, although baseline (pre-curcumin) imaging was not always presented [5]. More interestingly, the study also found that retinal Aß-plaque burden was reduced following an immune-based therapy, indicating a similar behaviour to the brain in response to the same therapy [82].

Near-infrared fluorescence (NIRF) ocular imaging has recently been explored for imaging of retinal biomarkers in Tg-AD mice (APP/PS1) [83]. Utilising the fluorescent probes CRANAD-X (to label Aß), and CRANAD-61 (to label reactive oxygen species; ROS), the authors found NIRF probes allowed differentiation of Tg-AD mice from WT mice and the detection of both soluble and insoluble Aß species. Moreover, some probes could be used to monitor therapeutic effects. Compared to NIRF brain imaging, NIRF ocular imaging could provide higher sensitivity for detecting Aß deposition, although the fluorescent signals were captured from the whole eye rather than specific tissues, such as the retina.

Topical endoscope fundus imaging was also applied in detecting early retinal amyloidopathy in Tg-AD mice ($APP_{swe}/PS1_{\Delta E9}$) [84]. A modified machine was assembled with a vision camera and tunable wavelength system to acquire monochromatic images across the visible to the near-infrared spectral range. Short-wavelength reflectance was reported to be significantly reduced over time in Tg-AD mice compared to WT mice. The optical changes in the Tg-AD mice were thought to be consistent with an increase in Rayleigh light scattering in the neural retina due to soluble Aß1-42 aggregates.

In addition to Aß, *in vivo* imaging of fibrillary tau in the retina of Tg-AD mice (P301S) was reported [40]. Cells containing fibrillary tau were labelled by the fluorophore FSB, administered systemically, and imaged *in vivo* using a modified Spectralis HRA+OCT system. Aggregated fibrillary tau was detected in the retina of aged P301S mice but no FSB-positive signal was observed in WT mice. Moreover, the number of FSB-positive cells in P301S mice consistently increased throughout observation (2-6.5 months). Interestingly, no FSB-positive cell loss was noticed following the formation of fibrillary tau, and the viability of neurons containing fibrillary tau was histologically confirmed by the neuronal marker NeuN at the end of the experiment. This indicated that the formation of fibrillary

tau alone may not be sufficient to cause neuronal death; a similar observation was made in the cerebral cortex [85].

Neuronal loss in the retina is histologically identified in the Tg-AD mice, along with Aß deposition and tau pathology. A recent study demonstrated for the first time that retinal ganglion cell death can be visualized *in vivo* in 3xTg-AD mice [86]. Using annexin V (Anx) and propidium iodide (PI) as cell death markers, significantly more RGCs were found to be undergoing the early phase of apoptosis (Anx+) and less necrosis (PI+) in the Tg-AD (14 months old), compared to WT controls (18 months old). Although a low level of apoptotic and necrotic cell death occurs as a correlate of normal ageing in mice, this study demonstrated a significant increase in the relative numbers of RGCs in early-phase apoptosis in the Tg-AD mice. To investigate whether oxidative stress in AD eyes influenced the phase of cell death, phorbol 12-myristate 13-acetate (PMA), an oxidative inducer, was intravitreally injected and RGC death imaged *in vivo*. PMA resulted in an increase in magnitude in the level of early apoptosis in Tg-AD mice compared to WT mice, and also a decrease in late apoptosis; a similar effect of PMA was found in a model of acute RGC death mediated by Aß [86].

Loss of retinal neurons can be reflected by reduced thickness in the retinal layers. By segmentation of retinal layers *in vivo* using OCT, a recent study demonstrated reduced RNFL/GCL thickness in a Tg-AD (TgCRND8) model compared to WT [42]. The *in vivo* observation that the number of RGCs significantly declined was confirmed histologically[42]. This is compatible with findings in AD patients, where significant thinning in the RNFL is reported and the changes in the RNFL thickness are correlated with disease severity of AD, suggesting the importance of OCT as a potential adjuvant in early diagnosis of AD [87][88], although it is disagreed with by others [89]. Nevertheless, OCT is the only *in vivo* imaging technique currently conducted in both humans and Tg-AD mice.

Electroretinography (ERG)

Retinal cell function has been evaluated by ERG recordings in Tg-AD mice. Scotopic threshold response (STR) represents an inner retinal function, and STR amplitudes were reported to be significantly reduced in Tg-AD ($APP_{swe}/PS1_{\Delta E9}$) mice, compared to WT counterparts [45]. In the same Tg-AD model, pattern ERG (P-ERG), representing the activity of RGCs, also showed a significant decline in amplitude [90]. Inner retinal function impairment was also found in other Tg-AD models, including 5xFAD mice [91] and P301S mice [92]. Using P-ERG and flash EGC (F-ERG) to assess inner and outer retinal function, respectively, a significant reduction was recorded in the P-ERG amplitudes while no changes were found in the F-ERG in 5xFAD mice, indicating that the inner retina but not outer retina undergoes functional decline [91]. A significant decline of P-ERG was also found in P301S tau transgenic mice, along with retinal acuity impairment [92]. The findings of the unaffected outer retina are consistent with other reports that cone function, recorded by photopic ERG, is preserved in aging Tg-AD (APP-PS1) [46] and that no difference in ERG responses are found between Tg-AD (APP-PS1) and WT in both dark-adapted (rod-pathway) and in light-adapted (cone-pathway) conditions [93], which is also the case in transgenic mice expressing human tau (hTau) [68]. Overall, the inner retinal function is affected in Tg-AD mice whereas outer retinal function remains preserved, although conflicting findings exist [37][94]. The inner retinal functional impairment could be associated with the enhanced level of Aß deposition [45].

THERAPEUTIC INTERVENTION IN Tg-AD

Although AD is the predominant cause of dementia worldwide [95], there are presently no treatments to cure it. The only drugs available on the market aim to delay the disease process with a very low success rate in some cases [96, 97]. Two classes of drugs are available: cholinesterase inhibitors and N-Methyl-D-aspartate (NMDA) receptor antagonists [98]. Despite being widely used, these drugs do not cure the disease and can be ineffective at slowing AD progression if started too late in the disease process. With discoveries highlighting the importance of the retina in AD, monitoring the efficacy rate of a drug using the retina as a window to the brain might become the new standard. In the following section, we are discussing new research targeting the Aβ cascade by inhibiting β- or γ-secretase, tau pathology, or immunotherapy focusing on the brain and the retina.

New drugs and natural compounds have been assessed *in vivo* to slow or reverse AD pathological processes in the brain. In this regard, 3xTg-AD mice (10-months-old) treated with white-wine polyphenol enriched water for two months have shown a reduction of Aβ40/42 and an increase of antioxidant molecules [99]. A study that treated young Tg2576 mice with a β-secretase inhibitor (TAK-070), administered orally, demonstrated a decrease of Aβ plaques and oligomers along with an increase of α-secretase activity, resulting in improved cognition [100]. Furthermore, in the same mouse model, Ibuprofen was shown to inhibit γ-secretase and reduce Aβ42 and Aβ plaques [101]. Another molecule, minocycline, decreased tau aggregation and hyperphosphorylation in a human tau transgenic mouse model [102]. However, the same drug was unable to decrease tau pathology in the 3xTg-AD mouse model despite being able to decrease Aβ plaque load [103].

Active and passive immunisation has also been widely used in transgenic mouse models. Active immunisation of PDAPP mice with Aβ42 resulted in an absence of Aβ plaques in young mice and a decrease of Aβ aggregation in older ones [104]. In the same mouse model, passive immunisation also reduced cortical Aβ plaque burden and Aβ oligomers in combination with cognitive improvement [105].

Taken together, these studies show that the brain has been widely and successfully used to assess the disease process in AD. However, the brain's localisation in the skull makes it almost impossible to monitor it non-invasively. Therefore, assessing therapeutic efficacy in the retina is of utmost importance and could pave the way to the development of novel biomarkers for AD in the future. Unfortunately, to date, few preclinical studies assessing treatment response in the retina of Tg-AD mice are available. These limited studies are summarised in Table 3.

One study (Koronyo-Hamaoui et al.), showed that active immunisation with modified myelin-derived antigens in APPswe/PS1δE9 mice once a month for three months resulted in a reduction of Aβ plaque number and size, in the retina and the brain, compared to controls [5]. Similarly, the immunisation of Tg2576 mice with Aβ oligomers or Aβ fibrils once a month for ten months resulted in a significant decrease of Aβ plaque burden in the retina and the brain. However, the immunisation resulted in an exacerbation of angiopathy and inflammation in the retina, characterised by microglial infiltration [106]. In another study with 5 month-old APPswe/PS1δE9 mice, the modulation of the immune system with a bone marrow transplant showed a reduction in retinal microglia, Aβ, and paired helical-filament tau, compared to age-matched wild-type controls [63]. In the same animal model, the intraperitoneal injection of memantine protected RGCs but did not show any difference in retinal layer thickness compared to controls [90]. Furthermore, the intravitreal administration of sNEP (a recombinant form of neprilysin which can cleave Aβ) in 5XFAD mice showed a reduction of Aβ40 and Aβ42 oligomers in the retina [107]. In another mouse model, TgCRND8, the intraperitoneal administration of an inhibitor of C-Jun N-terminal Kinase once a month for four months resulted in a reduction of Aβ oligomers and hyperphosphorylated tau, along with a decrease of retinal ganglion cell loss [42].

In other disease models, Ding et al. showed that passive immunisation with anti-Aβ in a transgenic mouse model of age-related macular degeneration resulted in a reduction of Aβ plaques in the retina, and this finding correlated with a reduction in the brain [108]. Likewise, in a rat glaucoma model, the intravitreal administration of Aβ antibodies resulted in less retinal ganglion cell loss [109, 110].

These promising findings suggest that the retina could be efficiently used to monitor Aβ deposition and treatment efficacy following different therapeutic strategies. However, further studies are needed to show a correlation with these data. Presently, the need for finding new biomarkers in AD is urgent, as shown by the failure of many clinical trials using therapeutic strategies, including β- or γ-secretase inhibitors and immunotherapy [111, 112]. These failures might be due to a wide spectrum of disease with patients with early and advanced disease enrolled into the same studies, whereas preclinical studies were mostly carried out on presymptomatic animals. The retina could potentially be used to diagnose AD earlier and start an early treatment which might foster the way for more successful clinical trials in the future.

TRANSLATIONAL IMPLICATIONS

Transgenic AD models are essential to understand AD pathogenesis and to perform preclinical assessments of novel therapeutics. However, the validity of relying on the transgenic models in clinical translations has been questioned due to the very high failure rate (~ 99.6%) of clinical trials of AD therapeutics that have been successful in preclinical testing in AD models [33, 97, 113, 114]. Several considerations are arising from the devastating clinical outcomes. Firstly, AD is a multifactorial disorder and the inherent complexity of AD is a major challenge for making transgenic AD models [35]. Currently developed AD models only mirror limited aspects of AD, replicating one or two specific pathological features of AD [113]. For example, most AD models develop only the amyloid plaques with a lack of other pathological features, such as neuronal loss and neurofibrillary tangles [14] [15]. Therefore, it is particularly important for researchers to understand the limitations associated with each model before carrying out preclinical evaluations. For example, transgenic mice expressing tau or tau/APP would be suitable models for preclinical trials of tau-oriented therapeutics [117], and those harbouring PS mutations might be more appropriate models for trials of γ-secretase modulators [116]. Whenever possible, potential therapies should be tested in multiple animal models, each of which exemplifies a unique aspect of AD pathology [34, 116]. New knock-in mouse models are being developed, and they are potentially more representative and are considered to be more physiological models of AD [34].

Additional to the limitations of specific pathological features of AD in each model, current transgenic models all harbour mutations associated with FAD while most clinical cases of AD are sporadic with the lack of a known genetic cause [33]. As such, therapeutic success in preclinical testing may not suggest that the therapeutics should be efficient in the sporadic form of human AD. Furthermore, the time course of disease in AD models is relatively shorter than human AD, and therapeutics that are efficient in animal models in the early disease course may not be necessarily successful in advanced human AD [117].

On the other hand, the failure of most clinical trials may reflect flawed hypotheses of AD or inadequate characterization of the preclinical pharmacodynamic and pharmacokinetic (PD/PK) properties [33, 113]. It can be also attributed to the ‘one drug, one target, one disease’ paradigm. Thus, endophenotype-based network medicine methodologies have recently been proposed to promote AD therapeutic development, by optimizing the usefulness of available data and supporting deep phenotyping of the patient heterogeneity for personalized medicine in AD [118].

Because of the limitations of AD models in clinical translation, more human-centric approaches have been developed and human tissue has been considered to have great potential for initial drug screening [34]. However, human tissue approaches cannot replace living animal models that allow testing the general toxicity of novel therapies and providing a whole-body system to assess the retina and brain in vivo.

Nonetheless, given the magnitude of the public health crisis associated with AD, we believe that the transgenic AD models will continue to play essential roles in testing novel therapeutics as well as providing insights into disease mechanisms.

CONCLUSION

With the global population ageing, the burden on quality of life resulting from dementia is ever increasing, while an early diagnosis of AD remains a big challenge. Animal models are providing fundamental information in searching for diagnostic biomarkers. Having similar pathological changes to the brain, the retina has become an increasingly attractive area for AD research. By imaging the retina non-invasively, AD biomarkers are likely to be detected early, before advanced progression occurs in the brain. In this respect, the eye can serve as a window into brain disease, facilitating early diagnosis, monitoring disease progression, and assessment of therapeutic efficacy in AD.

THE LIST OF ABBREVIATIONS

AD	Alzheimer's disease
Aß	amyloid-ß
NFT	neurofibrillary
CSF	cerebrospinal fluid
CNS	central nervous system
FAD	familiar Alzheimer's disease
Tg-AD	transgenic AD
INL	inner nuclear layer
ONL	outer nuclear layer
AC	amacrine cells
BC	bipolar cells
HC	horizontal cells
GCL	ganglion cell layer
RGC	retinal ganglion cells
ON	optic nerve
LGN	lateral geniculate nucleus
SC	superior colliculus
VC	visual cortex
mRGC	melanopsin RGC
SCN	suprachiasmatic nucleus
APP	amyloid precursor protein
PS1	presenilin-1
PS2	presunilin-2

PSEN	presenilin
GFAP	glial fibrillary acidic protein
OCT	optical coherence tomography
NIRF	near-infrared fluorescence
ROS	reactive oxygen species
Anx	annexin V
PI	propidium iodide
PMA	phorbol 12-myristate 13-acetate
ERG	electroretinography
STR	scotopic threshold response
NMDA	N-Methy1-D-aspartate

CONFLICT OF INTEREST

Professor M. Francesca Cordeiro is a named inventor on a patent application covering the DARC technology disclosed in this manuscript. The remaining authors declare no conflict of interest.

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