

Understanding Alzheimer's disease in Hong Kong: can regular retinal checkups be used to diagnose, monitor and study disease pathogenesis?

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Abstract

Recently, interest in using the retina to represent simple brain neurobiology has increased, particularly in regard to neurodegenerative disorders such as Alzheimer's disease. Various laboratories and clinics worldwide are currently evaluating the use of novel techniques to monitor retinal health in patients with Alzheimer's disease. Unfortunately many of these tools are still at the development stage, while others may be unavailable or expensive in the Asia Pacific region, including cities like Hong Kong. In this review we address the current state of Alzheimer's disease retinal research in Hong Kong, emphasizing the recent literature, the use of Alzheimer's disease mouse models in retinal/brain research, and the potential implementation of retinal checkups to diagnose and monitor neurodegeneration in patients with Alzheimer's disease. We believe that the combined efforts of local researchers and clinicians will not only advance our understanding of Alzheimer's disease pathogenesis in both the brain and retina, but

will also enhance the diagnosis and treatment options available for patients with Alzheimer's disease in Hong Kong.

Key words: Alzheimer disease; Amyloid beta-peptides; Retinal neurons; tau proteins; Translational medicine research

Introduction

Alzheimer's disease (AD) is recognized as the most common cause of dementia, a neurodegenerative disease affecting a continually increasing number of patients worldwide. In Hong Kong alone, dementia was estimated to affect approximately 103,433 people over the age of 60 years in 2009 and is expected to increase 222% by 2039.¹ It was also specified as the cause of an estimated 1112 deaths in 2014, up from just 999 in 2013 and 252 in 2001,² firmly placing it in the city's top 10 leading causes of death. Furthermore, of those diagnosed with dementia, approximately 65% are related to late-stage AD pathogenesis.³ Thus, as the size of the elderly population in Hong Kong is expected to increase up to an estimated 33% by 2064 (compared with 15% of the population in 2014⁴), the amount of interest in local AD

research and funding should also expand in order to advance our understanding of this debilitating disease and provide better patient care.

A vast amount of research has been published on the causes and symptoms of AD as well as possible treatment options and curative agents. Notably, even with our knowledge of the various risk factors, diagnosis of AD pre-mortem is limited.⁵ Clinicians currently rely on a list of AD criteria⁶ that in 85% to 90% of cases are enough to predict AD.⁷⁻⁹ A non-invasive technique to definitively provide a positive diagnosis would be invaluable, particularly for early detection. In response to this need, many laboratories have evaluated the use of positron emission tomography imaging^{10,11} and magnetic resonance imaging^{12,13} techniques to visualize the AD-induced changes that occur in the brain. These techniques, however, still do not properly address our lack of understanding about the causes of the disease, and many are expensive with limited availability in this region of the world. Furthermore, treatment options for these patients are also hindered by the inadequate number of specific drug targets.

Thus, it is the focus of several laboratories at the University of Hong Kong to address the underlying causes of AD by investigating AD-related retinal neurodegeneration in various animal models as a proxy for overall disease pathogenesis with the hope of not only identifying possible drug targets, but also developing potential diagnostic tools to use in conjunction with the current AD cognitive criteria. In this review, we have evaluated the potential use of regular retinal health examinations to monitor AD-related neurodegeneration in relation to AD prognosis in Hong Kong patients. We have briefly analyzed what is currently known concerning disease development and pathogenesis in both the brain and retina as well as the role of various AD animal models in our search for diagnostic biomarkers and treatment options.

The pathogenesis of Alzheimer's disease in the brain

AD is a progressive neurological disorder characterized by amyloid beta (A β) plaque and subsequent neurofibrillary tangle (NFT) formation in the brain.¹⁴⁻¹⁶ These pathological indicators are accompanied by decreased synaptic density, increased neuroinflammation, and neuronal cell death, ultimately leading to memory loss and learning deficits.¹⁷⁻²⁰ A β plaques build up in the brain as a result of excessive formation and reduced clearance of A β leading to extraneuronal aggregation.²¹⁻²³ The soluble oligomers formed are then thought to activate microglia- and astrocyte-related inflammatory responses, processes that subsequently cause oxidative stress, mitochondrial dysfunction and disturbed cellular homeostasis.²⁴⁻²⁶ NFTs are formed via the intraneuronal accumulation of hyperphosphorylated forms of the microtubule-associated protein tau.²⁷⁻²⁹ This type of extensive post-translational modification ultimately causes increased soluble tau, protein aggregation, and formation of NFTs that can block normal cellular transport.^{30,31} Tau

hyperphosphorylation and aggregation are likely triggered by upstream A β aggregation and appear to be required for A β -dependent cognitive dysfunction.³²⁻³⁶ Therefore, all AD patients are found on autopsy to have some level of A β and tau accumulation, and while the symptoms and progression of the disease vary greatly among individual patients, the level of A β aggregate- and NFT-induced neurodegeneration is likely related to the progressive decline in cognitive and functional ability observed during the life of the patient.^{12,37-39}

Notably, there are 2 types of AD: the sporadic age-related form (late-onset) and the genetic familial form (early-onset). Late-onset AD development has been linked to a wide range of genetic and environmental factors, including diet,⁴⁰ physical activity,^{40,41} microbiome,⁴² and obesity/cardiovascular diseases,⁴³ but the underlying causative mechanisms are largely unknown. In fact, late-onset AD patients are likely to have functional changes in their brain over 10 to 20 years before their manifestation.⁴⁴ This further obscures the key factors involved. Recent evidence suggests that there may be various genetic risk factors associated with sporadic AD. For example, both the ϵ 4 allele of apolipoprotein E (*APOE*)⁴⁵⁻⁴⁷ and the R47H allele of the triggering receptor expressed on myeloid cells 2 (*TREM2*)⁴⁸⁻⁵⁰ have been associated with a higher risk of developing AD. Although these genes have been linked to AD-related processes including amyloid clearance and blood brain barrier integrity, animal studies indicate that other genetic and/or environmental factors are necessary for the development of the full AD phenotype observed in humans.

Although the vast majority of AD patients are diagnosed with the late-onset form, close to 1% of cases are early-onset.^{51,52} The histological features of the A β plaques and NFTs, as well as the clinical symptoms to some extent, in early-onset AD are identical to those of the late-onset AD.⁵³⁻⁵⁶ Despite this, they appear to stem from different causative factors, with familial AD primarily involving mutations in amyloid precursor protein (*APP*),⁵⁷⁻⁵⁹ presenilin 1 (*PS1*),^{60,61} presenilin 2 (*PS2*),^{62,63} and/or tau genes.^{31,64} *APP*, a single transmembrane glycoprotein gene that is the precursor of A β , can be enzymatically cleaved by α -, β - and γ -secretases; but it is the sequential actions of the β -, and γ -secretases at the endoplasmic reticulum that generate the harmful AD-related form of A β .⁶⁵ Previous work has shown that mutations in the *APP* gene not only encourage cleavage by these secretases to generate more A β fragments, but they also increase their self-aggregation properties.⁶⁶ Alternatively, *PS1* and *PS2* are both multitransmembrane proteins that function in the γ -secretase complex.⁶⁷ Loss-of-function mutations in these genes also appear to promote A β generation and aggregation, with *PS1* mutations being more common in AD patients than *PS2* mutations.⁶⁸⁻⁷⁰ Finally, tau, encoded by the *MAPT* gene, is known to play an essential role in microtubule stabilization and the regulation of axonal transport processes in neuronal cells, with 6 isoforms being expressed in the central nervous system at different times during development.⁷¹⁻⁷⁷ Various missense and

alternative splicing mutations of the tau pre-mRNA appear to play a significant role in microtubule-binding ability, whereby increased levels of unbound tau may be due to the aberrant phosphorylation and self-aggregation observed in neurodegenerative diseases.^{78,79} Although some behavioral impairments have been observed,⁸⁰ tau knockout mice do not display any significant neurodegenerative defects.⁸¹

The pathogenesis of Alzheimer's disease in the retina

Although multiple ocular tissues may be involved in the vision deficits associated with AD,^{82,83} changes in the retina likely play a significant role. The retina develops from the neural tube, similar to other regions of the brain and central nervous system. While the mammalian retina has a unique physical structure, including 5 groups of neurons responsible for transmitting light signals to the brain (photoreceptors, bipolar cells, horizontal cells, amacrine cells and retinal ganglion cells [RGCs]), many of the signaling molecules, cytokines and immune responses in this tissue are similar to those utilized in the brain. Furthermore, visual deficits — such as problems with depth perception, reading, motion perception, color recognition and impaired spatial contrast sensitivity — are common in AD patients.⁸⁴⁻⁸⁹ These visual symptoms may be noticeable in the very early stages of AD, even prior to diagnosis, and have been linked with the level of cognitive decline in patients.^{19,90} These disturbances in an AD patient's vision have also been associated with a substantial loss of RGCs and decreased thickness of the retinal nerve fiber layer and macula as well as optic nerve degradation and decreased venous blood flow.⁹¹⁻⁹⁷ Further, amyloid plaques have also been detected in the retina of AD patients as well as patients with mild cognitive impairment.⁹⁸ Thus, it would appear these changes in the retina may mirror the extent of neurodegeneration in the brain during AD, making further investigation of these AD-linked retinal changes essential. Pathologic changes observed in the retina of AD patients appear to be closely related to those observed during glaucoma and age-related macular degeneration.⁹⁹⁻¹⁰¹ It is likely that common signaling pathways are functioning during these neurodegenerative processes; additional work is necessary to elucidate these factors.

There are numerous advantages using the retina to study AD pathology. For clinicians, the retina can be directly imaged in living patients without invasive measures, providing a novel means of diagnosing AD. For research scientists, using the retina to study AD has the added benefits of being easily accessible for macro-histologic analysis as well as single neuron imaging. Retinal tissue extraction is also relatively straightforward, allowing researchers to use standard gene expression techniques in addition to primary retinal cell culture for in-vitro studies of mechanisms and/or drug responses. A number of Hong Kong-based clinicians, in association with research scientists at the University of Hong Kong, have already applied various techniques, including optical coherence tomography (OCT), electroretinograms

(ERGs) and contrast vision testing, to evaluate retinal neurodegeneration in animal models as well as AD patients. These retinal examinations, however, are not yet used regularly in the clinical evaluation of AD.

Transgenic mice as a tool to study Alzheimer's disease-linked neurodegeneration

Numerous transgenic animals have been used to study brain development and disease. For AD alone, 118 mouse models have been created using single or multiple genetic manipulations resulting in various AD-related physiological changes.¹⁰² Most of these mouse models and studies focus on AD pathogenesis in the early-onset form because of their ease of use in terms of genetic manipulation and reproducibility, while late-onset/sporadic models are more challenging. As various review articles have already comprehensively summarized many of the transgenic mouse models currently in use,^{103,104} in the present review we focus on the use of 3 primary models (Tg2576, tau P301L, and 3xTg) to highlight the significant role of transgenic mice in AD research, particularly in Hong Kong-based laboratories.

Tg2576 mice

Tg2576 mice harbor a mutation in the *APP* gene (K670N/M671L) that was derived from a Swedish family with an extensive history of AD under the control of hamster prion promoter.^{105,106} These single mutation transgenic mice overexpress *APP* and have age/disease progression-related A β deposition, and the postmortem histology of the brain appears to similar to that of AD patients.^{107,108} Further, a reduction in the number of neurons was also detected, as were glial activation and increased expression of pro-inflammatory cytokines.¹⁰⁹⁻¹¹² Cognitive defects are also apparent, with age-dependent deficits in working and spatial memory as well as contextual and cued fear learning ability.^{106,113,114} Notably, the onset of AD appears to vary in this model, ranging from 6 to 10 months of age,¹¹⁵ and may be a factor of genetic background.

While Tg2576 mice model distinct AD symptoms, there is a noticeable difference in their magnitude. For example, the cognitive defects and neuronal cell loss are not as extensive as those observed in their human counterparts.¹¹⁶ *APP* transgenic mice also do not develop tau pathologies on their own, meaning that while these models may express hyperphosphorylated tau in some tissues, they do not present any of the downstream NFT-dependent defects observed in AD patients.¹¹⁷

tau P301L mice

To study the specific role of NFT formation in AD neuropathology, tau mutant mice have also been generated. One such model that expresses tau with a leucine replaced for the proline at position 301 (P301L)¹¹⁸ has shown to result in the production of NFTs in both the brain and spinal cord, resulting in significant spinal cord atrophy and a limited life expectancy.³⁰ Notably, in animals with late-onset spinal cord deterioration, NFT formation is observed to significantly

impair basic motor behavior.^{118,119} These findings are supported by those obtained using a conditional expression model, whereby the expression of tau P301L is controlled using a tetracycline expression system.¹²⁰ Tau pathology is rapid following tetracycline introduction, with NFTs forming as early as 2.5 months followed by a marked decrease in the number of neurons as well as spatial memory impairment in as little as 4 months.¹²⁰

As with most animal models, the limitations of tau P301L mice primarily stem from promoter-dependent expression bias (tissue/cell type localization as well as transgene expression levels/dose) in addition to the widespread effects of genetic background that can limit the use of this murine model for translational research. Furthermore, these animals did not form the hallmark A β plaques observed in AD patients,^{29,118} indicating that they do not mimic the full human AD phenotype.

3xTg mice

While the 2 previous mouse models allow AD-related amyloidosis and tau pathology to be separately investigated, in order to evaluate the combined effects of these processes, double and even triple transgenic mice have been produced. 3xTg mice, for example, incorporate the Swedish *APP* mutation, the tau P301L mutation, as well as a mutation in the *PS1* gene (M14V),³³ a combination that appears to result in phenotype changes that mimic human AD.¹²¹ Notably, while *PS1* homozygous knockout mice have lethal developmental defects in their central nervous system,¹²² mice harboring non-lethal mutations in the *PS1* gene do not appear to develop the same pathology as mice with *APP* mutations, although altered A β processing is evident.¹²³ These mice do not form A β plaques. Nonetheless, when used in conjunction with an *APP* mutation, the rate of amyloid deposition and plaque formation is accelerated.¹²⁴ Further, recent research has indicated that while increasing the expression and phosphorylation of tau has no effect on the development of A β aggregates, meaning NFT formation is likely a downstream event,¹²⁵ endogenous murine tau is necessary for *APP*-A β -mediated neurodegeneration.¹²⁶ Indeed, tau knockout mice appear to be resistant, at least in terms of cognitive decline, to A β -induced toxicity.¹²⁷

These findings indicate that in order to fully appreciate the full spectrum of AD pathogenesis, animal models presenting both A β plaque and NFT formation should be used. The triple transgenic 3xTg mice appear to be an ideal option, with the onset of AD pathology being observed at 3 months of age, beginning with A β deposition (mediated by the *APP* and *PS1* mutations) followed by tauopathy (mediated by the tau P301L mutation).^{32,128,129} The progressive development of plaques and tangles also appears to continue in an age-dependent manner and are accompanied by cognitive deficits as well as upregulated microglial activity.^{121,128,130-132} Although some neuronal loss was observed in these mice, this loss was not detected in the specific region of the hippocampus known to be the main area of cell loss in AD patients.^{133,134}

Retinal changes in Alzheimer's disease models

Importantly, many of the mouse models demonstrate AD-related visual dysfunction and retinal neurodegeneration.¹³⁵ For example, Tg2576 mice appear to have an age-dependent increase in both *APP* and A β deposition in various retinal cell types as well as a reduction in retinal thickness.^{136,137} These mice also appear to have increased expression of hyperphosphorylated tau and reduced axonal transport, although the formation of tangles is not described.^{138,139} An age-dependent decrease in retinal thickness is also observed in tau P301L mice, primarily in the peripheral region.¹⁴⁰ Alternatively, in the triple 3xTg AD mouse model, retinal A β plaques are observed in addition to glial activation.¹⁴¹

General limitations

Although there are many advantages using mouse models to mimic human AD, such as genome manipulation, controlled genetic background, rapid disease maturation, cost, etc., there are also a number of drawbacks. For example, most of the models are considered incomplete as they do not mimic the full spectrum of human AD pathogenesis, including the 3xTg mouse model, as they lack substantial cell and synaptic loss^{42,142} and appear to have slightly different biochemical and morphologic characteristics, particularly when compared with the sporadic form of AD.¹⁴³ Further, the use of inbred mouse strains eliminates interference from genetic background during laboratory investigation, but this is not an option when treating AD patients, whose genetic variability may play a significant role in the progression of the disease.¹⁴⁴ Nonetheless, these mouse models still offer the most direct means of studying AD outside of AD patients. In fact, the lack of complete AD-related structural changes and cognitive decline in these models¹⁴⁵ implies that they may better represent the prodromal phase of the disease, suggesting that they may be of the most value when evaluating upstream causative biomarkers as well as early diagnostic tools.¹⁴⁶ With this in mind, these models can be used not only to investigate early changes in gene expression and function in the brain, but also evaluate the role of the retina as a means to diagnose AD.

Translating basic science to clinical application

These transgenic models of AD have enabled researchers to potentially develop better treatment and diagnostic options for AD patients, particularly the use of retinal checkups as a proxy for evaluating AD-related neurodegeneration in the brain. Laboratory models allow testing of AD drugs prior to clinical trials; many animal models have retinal changes similar to those found in AD patients and are ideal for determining the applicability of various retinal scans, such as OCT, ERGs and contrast vision testing. These technological advances can elucidate the upstream biomarkers of this disease and detect expression and functional changes in the early stages of the disease prior to the irrevocable structural changes in both the retina and the brain. Discovering these biomarkers is useful in AD diagnosis and provides additional options for drug targeting during disease treatment.

While various conventional drugs — including acetylcholinesterase inhibitors, N-methyl-D-aspartate receptor antagonists, monoamine oxidase inhibitors, metal chelators, anti-inflammatory drugs and antioxidants^{147,148} — are often used in an attempt to treat or slow down the cognitive decline in AD patients, researchers have failed to produce a viable option for the treatment, cure and/or prevention of AD. Interestingly, a number of traditional Chinese medicines (TCMs) have recently been evaluated for their use as a therapy for AD and other dementias, either alone or in conjunction with other conventional drugs.^{149,150} Some of the naturopathic compounds found in TCMs have also been shown to protect and combat retinal neurodegeneration.^{151,152} The amount of active research in the field of TCM in the Asia Pacific region, including Hong Kong, in conjunction with the AD focus of various laboratories at the University of Hong Kong provides a foundation for continued research into natural AD treatment options. These researches evaluate these compounds in terms of their characteristics and in-vitro/in-vivo effects in local basic science laboratories as well as their translation to clinical use. The relationship between the University and the Hong Kong Alzheimer's Disease Association¹⁵³ also provides the opportunity for enhanced discussion between research scientists and clinicians in the search for optimal translational research models, both in vivo and in vitro, and ultimately the best treatment options for each patient.

Conclusion

The increasing prevalence of AD and the aging population in Hong Kong necessitate continued studies of this disease such as AD-related brain and retinal changes using both animal models and clinical subjects in order to develop additional diagnostic and treatment options. It is hoped that regular retinal health checkups will be used to diagnose, monitor and study AD in Hong Kong patients, and that new methodologies for drug development and AD diagnosis will be implemented in both basic science laboratories for AD animal models as well as in clinical settings in Hong Kong.

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Declaration

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