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● INVITED REVIEW

Alzheimer's disease: the silver tsunami of the 21st century

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Abstract

Alzheimer's disease (AD), a fatal progressive neurodegenerative disorder, has no cure to date. One of the causes of AD is the accumulation of amyloid-beta 42 (A β 42) plaques, which result in the onset of neurodegeneration. It is not known how these plaques trigger the onset of neurodegeneration. There are several animal models developed to (i) study etiology of disease, (ii) look for genetic modifiers, and (iii) identify chemical inhibitors that can block neurodegeneration and help to find cure for this disease. An insect model of *Drosophila melanogaster* has also provided new insights into the disease. Here we will discuss the utility of the *Drosophila* eye model to study Alzheimer's disease.

Key Words: Alzheimer's disease; neurodegeneration; amyloid plaques; amyloid hypothesis; animal models; *drosophila melanogaster*; *drosophila* eye

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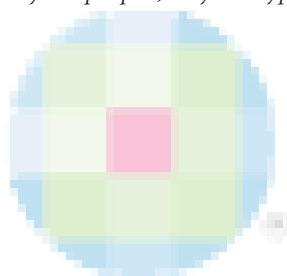
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Introduction

Alzheimer's disease (AD) represents a complex class of debilitating brain diseases that mostly affects people aged 65 and older. AD is characterized by progressive loss of neurons in the hippocampus and cortex, which results in the shrinkage of brain. Basically, the brain cells required to process, store and retrieve information are killed, which is characteristic of a neurodegenerative process (Singh, 2012). It results in decline in cognitive and behavioral functions like memory, thinking and language skills (O'Brien and Wong, 2010). AD, a common form of dementia, is a progressive neurodegenerative disorder, which killed half a million people in 2010. AD is aptly called as the silver tsunami of the 21st first century. It is the sixth leading cause of death in US. It is estimated that by 2050, the number of people with AD may nearly triple, from 5 million to as many as 16 million, barring the development of medical breakthroughs to prevent, slow or stop the disease (Xu, 2016). To date, there is no effective cure.

AD neuropathology is a proteinopathy, which is an outcome of misfolded proteins. AD is associated with two types of abnormal protein deposition in the human brain: (1) neurofibrillary tangles (NFTs) containing hyper-phosphorylated forms of a microtubule associated protein Tau, and (2) accumulation of the plaques of amyloid-beta (A β 42) peptide due to aggregation prone amyloidogenic domains (Hardy,

2009; Aguzzi and O'Connor, 2010; Fernandez-Funez et al., 2013; Selkoe and Hardy, 2016).

Generation of Amyloid-beta Plaques in AD

One of the hallmarks of AD is the accumulation of amyloid plaques in the brain. In the healthy brain these plaques are not present. The focus of this review is on the accumulation of A β 42 polypeptides formed by the improper cleavage of a transmembrane amyloid precursor protein (APP) in the brain (Figure 1) (Hardy, 2009; Aguzzi and O'Connor, 2010; O'Brien and Wong, 2010; Fernandez-Funez et al., 2013). Mutations in the gene encoding for APP have been linked to the familial form of AD. APP is proteolytically processed in the extracellular and intracellular domains by α and then γ -secretase enzymes, which leads to the generation of a forty amino acid long polypeptide (A β 40), which causes age-dependent learning defects but no neurodegeneration. However, the differential cleavage of APP by the activities of β - and γ -secretase enzymes lead to generation of forty two amino acid long polypeptide, hence A β 42 (Figure 1) (Hardy, 2009; Aguzzi and O'Connor, 2010; O'Brien and Wong, 2010; Fernandez-Funez et al., 2013; Selkoe and Hardy, 2016). These extra two amino acids cause the amyloid A β 42 polypeptide to become hydrophobic, resulting in formation of the amyloid plaques. Increased level of the more

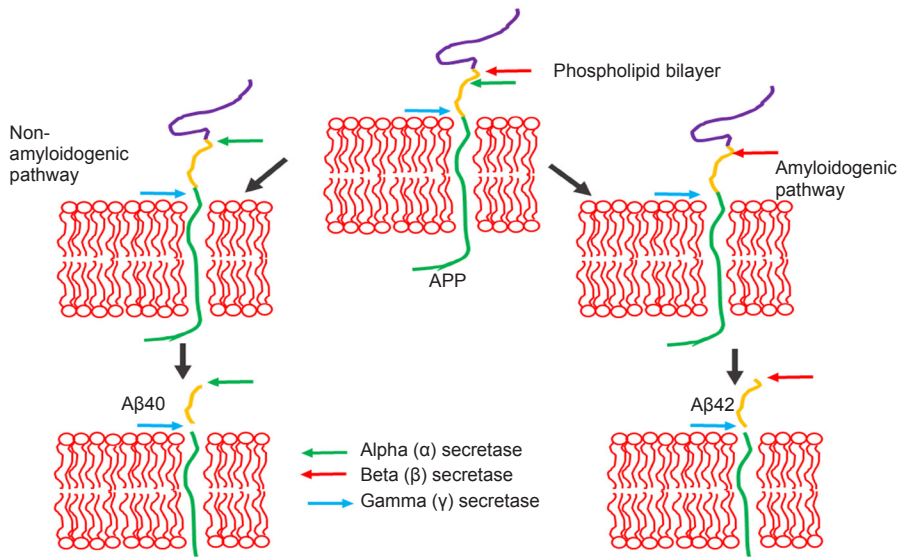


Figure 1 Schematic presentation of generation of amyloid-beta 42 (A β 42) plaques.

A β 42 are generated by improper cleavage of amyloid precursor proteins (APP). APP a transmembrane protein, is cleaved by alpha (α)-secretase and gamma (γ)-secretase to generate forty amino-acid (A β 40) long polypeptide. However, when APP is cleaved by beta (β)-secretase and gamma (γ)-secretase it generates forty two amino-acid (A β 42) long polypeptide. A β 42 polypeptide oligomerize to form a plaque.

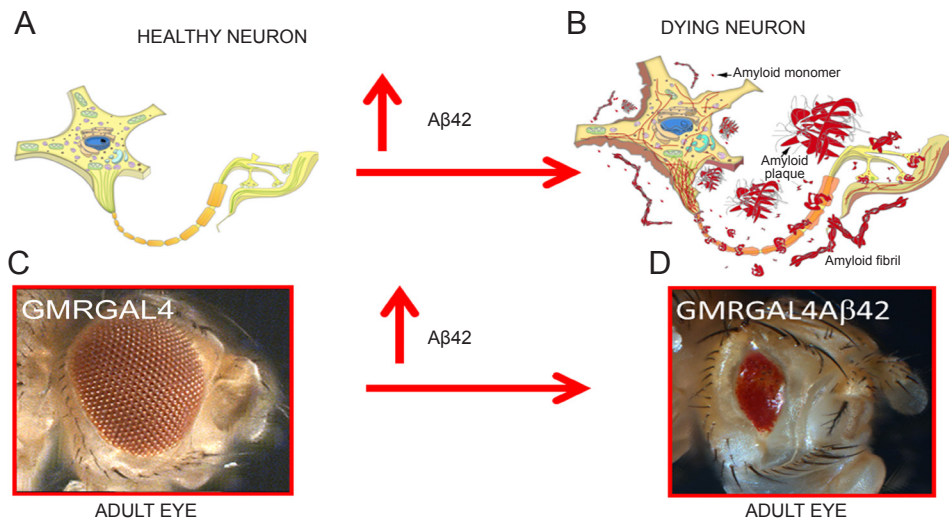


Figure 2 *Drosophila melanogaster* (fruit fly) eye model exhibits neurodegeneration due to accumulation of amyloid-beta 42 (A β 42) plaques.

Accumulation of A β 42 plaques in (A) healthy neuron triggers (B) death of a neuron. (C, D) Misexpression of human A β 42 in healthy photoreceptor neurons of *Drosophila* eye using transgenic approach causes a (C) wild-type adult compound eye to change into a (D) highly reduced adult eye due to induction of neuronal cell death (Tare et al., 2011).

toxic form of human A β 42 polypeptide is responsible for the neuropathology seen in AD. These plaques disrupt normal cellular processes through oxidative stress and aberrant signaling, resulting in the loss of synaptic activity and death of neurons.

Even though many gene mutations responsible for AD have been identified, the detailed genetic signaling mechanism(s) responsible for this neurodegeneration and progression of AD remain indefinable. A great deal of efforts are channeled towards discerning the underlying mechanisms that induce A β 42 metabolism and the pathways (e.g., apoptosis and autophagy) that trigger neuronal cell death. The rationale is to identify the molecular genetic mechanism of generation of amyloid (A β 42) accumulation, which will allow (a) development of biomarkers for early detection of AD, and (b) development of strategies to prevent accumulation of amyloid plaques, thereby leading to identification of drug targets for AD. These insights can lead to a cure or better therapeutic strategies for this disease.

Animal Models for AD

Clinical trials largely aimed at removing these A β 42 plaques have not yet been efficient. A β 42 mediated neurodegeneration is a dominant gain-of-function phenotype. Misexpression of human A β 42 polypeptides trigger neurodegeneration in traditional mammalian models like the Mouse (*Mus musculus*) as well as in the genetically tractable alternative models like zebrafish (*Danio rerio*), roundworm (*Caenorhabditis elegans*), and the fruit fly (*Drosophila melanogaster*) (Iijima and Iijima-Ando, 2008; Moloney et al., 2010; Harvey et al., 2011; Singh and Irvine, 2012). It has been seen that the primary structures of the A β peptide in rats and mice differ from their human counterpart at three amino acid residues. Furthermore, the complete A β -peptide profile produced by the processing of APP in these rodents exhibits major differences in comparison to that of humans. In guinea pigs, the rabbit A β peptide sequence is identical to human but does not present AD pathology spontaneously. Therefore, alternative animal models for AD have been

developed to understand the mechanism(s) of regulation of human A β 42 protein accumulation, and identification of the signaling pathways (e.g., cell death) involved to generate insights into the etiology of the disease (Bier, 2005; Singh and Irvine, 2012; Moloney et al., 2010; Pandey and Nichols, 2011; Tare et al., 2011).

Using an insect *Drosophila melanogaster* (a.k.a. fruit fly), to model human disease is beneficial for many reasons (Bier, 2005). Most importantly, their entire genome is sequenced, and is highly conserved with humans and is significantly less redundant (Lenz et al., 2013). Furthermore, they are smaller in size and thus can be stored efficiently, are cost effective, and can produce two generations in a month (Bier, 2005; Singh and Irvine, 2012; Lenz et al., 2013). It makes *Drosophila* an ideal model for studying age related progressive diseases like AD. Several models of AD have been developed in *Drosophila*. These include transgenic flies misexpressing human A β 42, human A β 40, human APP, BACE, β -secretase and *Drosophila* Psn, FAD (Cauchi and van den Heuvel, 2006; Cao et al., 2008; Iijima and Iijima-Ando, 2008; Tare et al., 2011; Fernandez-Funez et al., 2013). These flies display several aspects of clinical AD neuropathology and symptoms including the generation of amyloid aggregates, external morphological abnormalities, dramatic neuroanatomical changes and defects in motor reflex behavior and memory. As a proof of concept, treatment with a γ -secretase inhibitor was shown to suppress these phenotypes. *Drosophila* has also proved informative as a model of other neurodegenerative diseases like Parkinson's disease, Huntington's disease, fronto-temporal lobar degeneration (FTLD), and amyotrophic lateral sclerosis (Bier, 2005; Pandey and Nichols, 2011; Singh and Irvine, 2012; Fernandez-Funez et al., 2013).

***Drosophila* Eye as Disease Model System**

Drosophila has a fully functional nervous system with a structural design that separates specialized functions such as vision, olfaction, learning and memory. The *Drosophila* eye develops from a simple monolayer epithelium called the eye-antennal imaginal disc which grows during larval stages to form a highly organized compound eye comprised of photoreceptor cells, cone cells, and pigment cells (Ready et al., 1976; Kumar, 2011; Singh et al., 2012; Kumar, 2013; Tare et al., 2013). The precise structure of *Drosophila* eye makes it very sensitive to genetic disruptions/manipulations and it can be easily screened using a stereo microscope for phenotypes generated in the eye field. Thus, *Drosophila* eye allows quick screening of large sample size. The *Drosophila* model has a repertoire of tools that make it indispensable, including the ability to express foreign genes along spatio-temporal axes that can mimic several important neurodegenerative disorders in the compound eye (Bier, 2005; Singh and Irvine, 2012). Adding to this, the eye is not essential for the viability or fertility of the fly. Therefore, the *Drosophila* eye is an ideal organ system to assay the effect of neurodegeneration. Furthermore, the genes involved in eye development are structurally and functionally similar between insects and humans. The main advantage of the *Drosophila* eye is the ability to

directly visualize the cellular and developmental defects induced by neurodegeneration causing agents.

We have utilized *Drosophila* eye to model A β 42 mediated neurodegeneration. Using Gal4/UAS transgenic systems (Brand and Perrimon, 1993), we misexpressed human A β 42 in the differentiating photoreceptor neurons of the developing eye (Tare et al., 2011; Moran et al., 2013; Steffensmeier et al., 2013; Cutler et al., 2015). It resulted in progressive neurodegenerative phenotype; Cutler et al., 2015 in the *Drosophila* eye, which is similar to AD like neuropathology (Figure 2). However, *Drosophila* model has some challenges too. In comparison to the vertebrates, *Drosophila* has less complex and adaptive immune system. Furthermore, effects of drugs on the organism might differ strongly (Prussing et al., 2013).

***Drosophila* Model for Genetic Screens**

Drosophila model has been extensively used to screen (i) for genetic modifiers responsible for onset and manifestation of disease and (ii) chemical libraries to look for therapeutic targets to find cure for the disease. *Drosophila* model with the repertoire of genetic tools allows several different approaches like a forward genetic screens, which involve a random mutagenesis via chemicals or X-ray radiation and then to identify the genes responsible for that phenotype, or reverse genetic screen where you mutate a gene and then look for the phenotype caused by loss-of-function of that gene (Bier, 2005; Singh and Irvine, 2012). Forward genetic screens are decidedly useful as evidenced by the Nobel prize awarded to C. Nüsslein-Volhard, E. Wieschaus and E.B. Lewis in 1995 for their work in identifying genes involved in early fruit fly development (Nusslein-Volhard and Wieschaus, 1980; St Johnston, 2002; Lenz et al., 2013). However, mapping of mutations using a classical genetic approach can be time consuming. The advent of new genetic tools, like single nucleotide polymorphisms (SNPs), has alleviated some of this problem in forward genetic screens (Lenz et al., 2013). Despite this, few strictly forward genetic screens have been undertaken in an AD background using a *Drosophila* model. In reverse genetic screens, a known gene is disrupted, typically utilizing the Gal4/UAS system (Brand and Perrimon, 1993) to misexpress a gene or silence a gene using RNA interference (RNAi) (Lenz et al., 2013).

Modifier (also known as enhancer or suppressor) screens are highly valuable because they combine the advantages of both forward and reverse genetics and carried out using easily assayable phenotypes (Lenz et al., 2013; Prussing et al., 2013). In AD, the A β 42 neurodegenerative phenotype and the rough eye phenotype are easily assayable phenotypes (Tare et al., 2011). A *Drosophila* eye model was established where A β 42 is misexpressed in the developing fly eye results in highly reduced neurodegenerative eye (Cao et al., 2008; Iijima and Iijima-Ando, 2008; Tare et al., 2011). We employed this model for a small scale modifier screen to look for the genetic modifiers which can either enhance/suppress the AD eye phenotype by increasing levels of highly conserved signaling pathways (one at a time) (Tare et al., 2011; Moran et al., 2013). Using this screen, we have identified the homeotic

gene *teashirt* (*tsh*), highly conserved apical basal polarity marker Crumbs (*Crb*) (Steffensmeier et al., 2013) as modifiers of A β 42 mediated neurodegeneration, making these genes as possible target for AD therapies.

The similar logic was utilized for several high throughput screens (HTS) as well as smaller screens to identify genetic modifiers of AD. Though many genetic screens for modifiers of Tau induced neurodegeneration have been carried out, few large-scale screens have investigated modifiers of the A β 42 neurodegenerative phenotype (Lenz et al., 2013; Prussing et al., 2013). Several other earlier genetic screens to identify modifiers of the A β 42 phenotype have resulted in a list of potential downstream genetic targets (Cao et al., 2008). Tan et al. used a screen to identify loss of function mutations that either suppressed or enhanced the A β 42 neurodegenerative phenotype (Tan et al., 2008). Using a IInd and IIIrd chromosome deficiency kit they identified a deficiency that uncovers *Toll* (*Tl*) suppresses the A β 42 mediated neurodegeneration. Binding of the Toll extracellular domain initiates a signaling cascade that eventually leads to activation the NF κ B signaling, a pathway which plays a role in innate immunity and inflammatory responses. Tan et al. (2008) identified this inflammatory signaling as playing a key role in promoting neurodegenerative processes.

Ultimately, the goal of these types of genetic screens is to identify a particular protein or signaling pathway member, which is involved in AD mediated neuropathology that can finally be targeted with a chemical compound (Pandey and Nichols, 2011). Identification of such a chemical compound is typically accomplished by *in vitro* HTS of a chemical library. Once a compound is identified, it is optimized for human usage through a series of tests in animal models. This process can be inefficient when utilizing traditional *in vitro* cell cultures or biochemical assays. Furthermore, the paradigm in therapeutic treatments has shifted from “one disease-one target” to an understanding that many diseases result from a variety of factors and interactions. Based on these two problems with traditional methods in drug development, *Drosophila* has emerged as a valuable model (Pandey and Nichols, 2011).

It has already been noted that *Drosophila* as a model is space, time and resource efficient (Lenz et al., 2013). In terms of drug development, flies present an advantageous supplement or alternative to traditional methods because they allow chemical compounds to be tested *in vivo* in a whole organism as opposed to an isolated culture (Pandey and Nichols, 2011). Though considerations regarding the discrepancies between flies and humans in pharmacokinetics, pharmacodynamics and the toxicity of particular drug administered in *Drosophila* are necessary, overall the model has emerged as highly useful in secondary post screening validation. Even though other disease systems have utilized *Drosophila* as primary screening models, there are currently no studies which have utilized *Drosophila* as a primary screening model for novel drug discovery in AD (Pandey and Nichols, 2011).

Drug administration typically takes the form of solid media to which drugs are added and larvae are allowed to

grow on. Though *Drosophila* are useful for HTS, the amount of chemicals screened is significantly lower, typically 500 to 1,000 small molecules per month, than traditional cell culture assays, which can screen as many as 10,000 small molecules per month. Despite this, screens utilizing flies typically result in a higher proportion of hits with therapeutic value. In AD models like *Drosophila*, traditional HTS are frequently utilized first to take advantage of their efficiency and identified small molecules are then tested in flies (Pandey and Nichols, 2011).

One such screen to utilize *Drosophila* as secondary post screening validation identified a particular small molecule that inhibited A β peptide aggregation. Using a traditional HTS based on A β 42-GFP fusion they identified an inhibitor of A β aggregation. The most effective inhibitor, D737, was administered to transgenic flies with A β 42 misexpressed in neural tissues. They found that not only did the compound reduce the toxicity of A β 42 oligomers, but that it also increased the lifespan of the A β 42 transgenic flies and their locomotive activity (McKoy et al., 2012).

A recent study examined the neuroprotective effect of a flavonoid derivative, 2-(4' Benzloxyphenyl)-3-hydroxychromen-4-one, on the rough eye phenotype of transgenic A β 42 flies (Singh et al., 2014). The compound was identified using an *in silico* docking approach, then administered to transgenic A β 42 flies to test *in vivo*. Approximately 70% of A β 42 transgenic flies showed a rescued eye phenotype and there was a significant reduction in plaques in A β 42 transgenic larvae (Singh et al., 2014). While this type of post screening validation is certainly valuable, other disease models, including epilepsy and Fragile X syndrome (Stilwell et al., 2006), have taken advantage of *Drosophila* as a primary screening tool.

Conclusion

Here the vast contribution and potential of the *Drosophila* eye as a model for understanding the cellular and molecular mechanisms of neurodegeneration was explored. The eye model can be exploited for initial screening of the genetic modifiers or chemical inhibitor targets, as well as candidate validation. The *Drosophila* eye as a validation tool in drug discovery has gained momentum, which allows identification of compounds that would likely be effective in mammalian models and humans. Genetic and pharmacological screens in the fly eye will serve as a strong screening tool for therapeutic approaches in the future.

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