Received: 11 October 2023 | Revised: 28 January 2024 | Accepted: 29 January 2024

DOI: 10.1111/ahg.12554

Annals of human genetics WILEY

REVIEW

Using *Drosophila* amyloid toxicity models to study Alzheimer's disease

Elli Tsintzas | **Teresa Niccoli**

Department of Genetics, Evolution and Environment, Institute of Healthy Ageing, University College London, London, UK

Correspondence
Teresa Niccoli, Department of Genetics, Evolution and Environment, Institute of Healthy Ageing, University College London, London, UK. Email: t.niccoli@ucl.ac.uk

Funding information
Alzheimer's Research UK; Dunhill Medical Trust; UK Research and Innovation

Abstract

Alzheimer's disease (AD) is the most prevalent form of dementia and is characterised by a progressive loss of neurons, which manifests as gradual memory decline, followed by cognitive loss. Despite the significant progress in identifying novel biomarkers and understanding the prodromal pathology and symptomatology, AD remains a significant unmet clinical need. Lecanemab and aducanumab, the only Food and Drug Administration approved drugs to exhibit some disease-modifying clinical efficacy, target Aβ amyloid, underscoring the importance of this protein in disease aetiology. Nevertheless, in the absence of a definitive cure, the utilisation of preclinical models remains imperative for the identification of novel therapeutic targets and the evaluation of potential therapeutic agents. *Drosophila melanogaster* is a model system that can be used as a research tool to investigate neurodegeneration and therapeutic interventions. The short lifespan, low price and ease of husbandry/rearing make *Drosophila* an advantageous model organism from a practical perspective. However, it is the highly conserved genome and similarity of *Drosophila* and human neurobiology which make flies a powerful tool to investigate neurodegenerative mechanisms. In addition, the ease of transgenic modifications allows for early proof of principle studies for future therapeutic approaches in neurodegenerative research. This mini review will specifically focus on utilising *Drosophila* as an in vivo model of amyloid toxicity in AD.

KEYWORDS

Alzheimer's disease, amyloid beta, amyloid precursor protein, *Drosophila melanogaster*, GeneSwitch

1 | INTRODUCTION: THE GLOBAL CHALLENGE OF ALZHEIMER'S DISEASE

Ageing represents the primary risk factor for developing Alzheimer's disease (AD), with approximately 96% of AD cases occurring in individuals aged 65 and above (Padmanabhan & Götz, 2023). AD stands as the most prevalent cause of dementia and presents a significant and unmet medical challenge (Padmanabhan & Götz, 2023). Dementia encompasses a range of disorders characterised by cognitive function deficits. AD is primarily characterised by memory loss and progressive cognitive

decline, although it can involve impairments in speech and visuospatial processing (Knopman et al., 2021). Disease progression is characterised by a general physical decline in which eventually the abilities to perform basic bodily functions, such as walking, swallowing and speaking, are lost. Patients live an average of 8 years following diagnosis but can live up to 20, requiring around-the-clock care in the end stages of disease (Alzheimer's Association, 2020). Globally, in 2019, the cost of dementia was estimated to be $2.8 trillion (Nandi et al., 2022). As AD is closely linked to the aging process, as global life expectancy continues to rise due to advancements in healthcare, the prevalence of AD is expected to increase accordingly, with cases almost tripling by 2050 (Nichols et al., 2019). In the United Kingdom, dementia and AD were responsible for the majority of deaths in 2022 (Death registration summary statistics, England & Wales, 2022). Specifically, they accounted for 65,967 deaths. Currently, there are no therapeutic strategies available that can halt the course of the disease or reverse its progression, although recent clinical trials have shown some initial promise.

2 | THE GENETICS AND PATHOPHYSIOLOGY OF AD

AD is a proteinopathy disorder characterised by the accumulation of intracellular neurofibrillary tangles (NFTs) composed of hyper-phosphorylated Tau (p-Tau) and extracellular plaques of toxic amyloid-β (Aβ) peptides, the most common being Aβ1–42 (Padmanabhan & Götz, 2023). This leads to progressive neurodegeneration, with seminal studies indicating that neuronal cell death is initiated in the entorhinal cortex (Braak & Braak, 1992; Van Hoesen et al., 1991), propagating into the hippocampus and then spreading to the rest of the brain (Braak & Braak, 1991). However, recent studies have implicated pathological changes in the locus coeruleus as an even earlier occurrence in the disease course (Grudzien et al., 2007; Theofilas et al., 2017).

2.1 | Aβ toxicity

There are two forms of disease, a common late onset sporadic form (LOAD) and an early onset familial form (EOAD), with a mean age-of-onset below 60 (Kumar-Singh et al., 2006). Three genes carry autosomal dominant variants leading to EOAD: amyloid beta precursor protein (APP), presenilin 1 (PSEN1) and presenilin 2 (PSEN2) (Bekris et al., 2010). All the familial variants cause increased misprocessing of APP. This led to the formulation of the amyloid hypothesis, which states that AD is caused by the misprocessing of APP and the increased production of neurotoxic Aβ1–42 peptides (Haass & Willem, 2019) (Figure 1), (Rosenberg et al., 2016). APP is mostly cleaved sequentially by α-secretase and then by γ-secretase (a complex consisting of four individual proteins, including PSEN1 and PSEN2) leading to the formation of Aβ1–40 peptides. However, in AD, there is a significant upregulation of β-secretase (also known as β-site APP cleaving enzyme [BACE]) cleavage, leading to the formation of Aβ peptides that are 42 residues long (Aβ1–42). The inclusion of two additional hydrophobic residues in Aβ1–42 leads to the formation of β-sheet-rich structures, which further aggregate as neurotoxic plaques in the brain (Chen et al., 2017; Lemere et al., 1996; Quartey et al., 2021).

Approximately 30 pathogenic APP variants have been reported (Li et al., 2019), and these generally increase Aβ1–42 toxicity. For example, the Swedish (KM1670/671NL) variant is located at the β-cleavage site of APP resulting in increased β-secretase cleavage and thus increased Aβ1–42 production (Mullan et al., 1992; Rosenberg et al., 2016). The arctic variant (E693G) is within the Aβ region of APP and induces Aβ1–42 aggregation and increased plaque deposition compared to wild-type Aβ1–42 (Cheng et al., 2004; Lu et al., 2019; Nilsberth et al., 2001). In addition, Arctic Aβ1–42 exhibit increased intracellular toxicity and resistance to intracellular degradation compared to wild-type Aβ1–42 (Lu et al., 2019). PSEN1 and PSEN2 are components of the γ-secretase enzyme complex alongside nicastrin, PSEN enhancer 2 and anterior pharynx defective 1A (APH-1) and APH-2 (Kimberly et al., 2003). The presenilins are part of the component responsible for APP cleavage in the γ-secretase complex (De Strooper et al., 1998; Steiner et al., 1999), and PSEN1 variants are the most common cause of familial EOAD, whereas variants in PSEN2 and APP are much rarer (Bekris et al., 2010).

In addition to causative variants, over 600 genes have been identified as increasing susceptibility of developing AD (Knopman et al., 2021). A recent 2022 genome-wide association study (GWAS) identified 75 risk loci for AD with 42 being novel at the time of the study (Bellenguez et al., 2022).

Sporadic inheritance of the ε4 isoform of apolipoprotein E (APOE) is considered the most prominent genetic risk factor of LOAD (Bekris et al., 2010) and has been demonstrated to increase amyloid burden in the brains of AD patients (Castellano et al., 2011). Homozygote carriers of the APOE ε4 allele have an increased risk of developing AD 12–15 times more compared to APOE ε3 carriers (Van Der Lee et al., 2018). In addition, APOE ε4 carriers are associated with an earlier age of AD onset, possibly as a result of accelerated Aβ1–42 accumulation in the brain (Bales et al., 1999; Castellano et al., 2011). Although the exact neurobiological mechanism has not been elucidated, in vivo studies point to a potential impairment in Aβ1–42

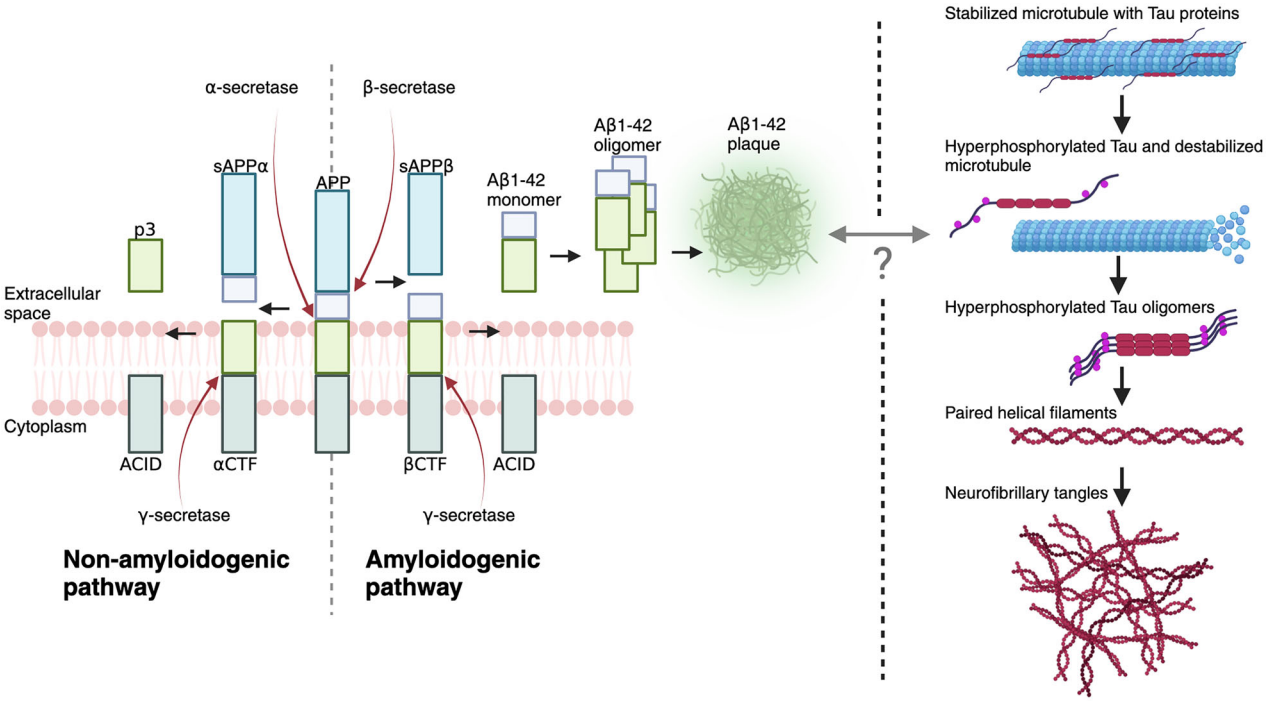

FIGURE 1 Amyloid toxicity and neurofibrillary tangle (NFT) pathology. Proteolytic processing of amyloid precursor protein (APP) can be amyloidogenic or non-amyloidogenic. Aβ1–42 peptides are generated from the cleavage of APP by secretases. α-Secretase drives the non-amyloidogenic pathway, whereas β-secretase drives the amyloidogenic pathway. α-Secretase cleaves APP into APPsα and αCTF (C-terminal fragment), and αCTF is further cleaved by γ-secretase to produce ACID and p3. In contrast, β-secretase cleaves APP into APPsβ and βCTF, and γ-secretase cleaves βCTF to generate Aβ1–42 and ACID. However, it is the imbalance of proteolytic processing favouring the β-secretase-mediated amyloidogenic pathway which is the predominant pathogenic factor. Although the cleavage product p3 from the non-amyloidogenic pathway appears to be nontoxic, the monomeric Aβ1–42 peptides generated from the amyloidogenic pathway have the propensity to aggregate and form neurotoxic oligomers. Tau is a microtubule stabilising protein, but certain toxic isoforms can undergo hyperphosphorylation. p-Tau exhibits reduced ability to bind to tubulin, resulting in reduced stability of microtubules. As a consequence, p-Tau monomers accumulate and oligomerise, eventually assembling into fibrous paired helical filaments, which give rise to the formation of neurotoxic neurofibrillary tangles (NFTs). The precise relationship between amyloid toxicity and the pathogenesis of NFTs is not yet fully understood. AICD, amyloid precursor protein intracellular domain; APP intracellular domain; APP, amyloid precursor protein; APPsα, secreted amyloid precursor protein-alpha; APPsβ, secreted amyloid precursor protein-beta; Aβ, amyloid beta; NFT, neurofibrillary tangle; p-Tau, hyperphosphorylated Tau αCTF, alpha C-terminal fragment; βCTF, beta C terminal fragment. *Source*: Created with BioRender.com.

clearance (Bales et al., 1999). Castellano et al. (2011) highlighted the most common genes, and their fly orthologues, in which variants are known to cause familial EOAD and the most common genes in which variants are risk factors for sporadic LOAD (Table 1).

Unsurprisingly, targeting the amyloidogenic APP processing has been a significant target for therapeutic interventions (Karran & De Strooper, 2022). The developments of monoclonal antibodies (mAb) against various forms Aβ, including monomers, protofibrils and plaques, have exhibited initial promise as the first disease-modifying treatments available for AD (Budd Haeberlein et al., 2022; Swanson et al., 2022; van Dyck et al., 2022). Aducanumab, a mAb targeting the aggregated Aβ in amyloid plaques, and lecanemab, a mAb targeting soluble Aβ protofibrils, have recently been approved by the Food and Drug Administration (FDA) for the treatment of AD (Cavazzoni, 2021)). In particular, lecanemab in a large phase 3 trial demonstrated a slower cognitive decline at 18 months, compared to the placebo group (van Dyck et al., 2022); however, it also led to severe adverse events in some participants (Budd Haeberlein et al., 2022; Swanson et al., 2022; van Dyck et al., 2022). Although both these mAb are not cures, for the first time, they demonstrate that interventions targeting Aβ can modulate disease progression, proving that indeed Aβ is a driver of disease, and validating the use of Aβ toxicity models for AD research.

2.2 | The link between Aβ and Tau

p-Tau aggregates in the brain and can lead to the formation of neurotoxic NFTs (Kondo et al., 1988) (Figure 1) which promote neurodegeneration through the disruption

TABLE 1 A summary of the genes, and their fly orthologues, in which pathogenic variants are known to cause familial EOAD and the most common genes in which pathogenic variants are risk factors for sporadic LOAD.

Gene	Description	Implicated core biological processes	Fly orthologue with % similarity to the human gene
APP	Variants cause autosomal dominant forms of EOAD (familial). i.e. Swedish (KM1670/671NL)	Neural progenitor cell proliferation regulator (Caillé et al., 2004). Learning and memory (Bourdet et al., 2015)	*APP-L* 24%
PSEN1	Variants cause autosomal dominant forms of EOAD (familial). i.e. M146I	Proteolytic processing of APP and Notch signalling pathway components (De Strooper et al., 1998; Steiner et al., 1999).	*Psn* *PSEN1* 47%
PSEN2	Variants cause autosomal dominant forms of EOAD (familial). i.e. N141I	Proteolytic processing of APP and Notch signaling pathways components (De Strooper et al., 1998; Steiner et al., 1999).	*Psn* *PSEN2 46%*
APOE	Sporadic inheritance of the ε4 isoform of APOE is a genetic risk factor of LOAD	Component of plasma lipoproteins, involved in their production, conversion, and clearance (Husain et al., 2021).	Not conserved
TREM2	Inheritance of certain *TREM2* variants correlates with an increased risk of LOAD development. i.e. R47H	Receptor is believed to promote mTOR signalling and sustaining microglial function (Ulland & Colonna, 2018).	Not conserved
PICALM	Single nucleotide variants of *PICALM* are associated with increased risk of LOAD development. i.e. rs3851179	Protein has a role in clathrin-mediated endocytosis (Tebar et al., 1999).	*Lap* 37%
ABCA7	Single nucleotide variants of *ABCA7* are associated with increased risk of LOAD development. i.e. rs3764650	Transporter is implicated in lipid metabolism and trafficking (Dib et al., 2021).	Not conserved
BIN1	Certain *BIN1* variants are associated with in with increased risk of LOAD development. The specific expression levels of variants are not fully elucidated	Protein is implicated in a variety of fundamental cellular processes including membrane trafficking, endocytosis, and regulation of cytoskeleton dynamics (Chapuis et al., 2013).	*Amph* 28%
CLU	Single nucleotide variants of *CLU* are considered a genetic risk factor for increased risk of LOAD development i.e. rs11136000	Clusterin protein functions as an extracellular chaperone that plays a role in the clearing of misfolded proteins (Rodríguez-Rivera et al., 2021)	Not conserved
CR1	Single nucleotide variants of CR1 are associated with increased risk of LOAD development. i.e. CR1-B allele	Receptor acts as a regulator of complement activation which is involved in immune system function (Khera & Das, 2009).	*Hasp* 22%

Abbreviations: Aβ, amyloid beta; ABCA7, ATP binding cassette subfamily A member 7; Amph, Amphiphysin; APOE, apolipoprotein E; APP, amyloid precursor protein; APP-L, amyloid precursor protein-like; BIN1, Bridging integrator 1; CLU, clusterin; CR1, Complement C3b/C4b Receptor 1; EOAD, early onset Alzheimer's disease; Lap, like-AP180; LOAD, late onset Alzheimer's disease; mTOR, mechanistic target of rapamycin; PICALM, phosphatidylinositol binding clathrin assembly protein; PSEN1, presenilin 1; PSEN2, presenilin 2; Psn, presenilin; TREM2, triggering receptor expressed on myeloid cells 2.

of multiple processes. The association between Aβ1–42 and Tau pathology in relation to AD pathogenesis remains unclear but according to the amyloid hypothesis, p-Tau NFTs are downstream of the amyloid cascade. The first in vivo evidence emerged in 2001 when *Science* published two sequential articles that demonstrated that the injection of Aβ1–42 (Mikol et al., 2001) and a pathogenic variant of APP (Lewis et al., 2001) induced NFT pathology in mice (Bloom, 2014). Further in vivo studies have suggested that Tau pathology is a requisite of Aβ1–42 toxicity since eliminating Tau in murine models carrying Pathogenic variants of APP protected against amyloid neuronal toxicity (Leroy et al., 2012; Roberson et al., 2007). Additionally, a study with human AD patients reported that the presence of cortical Tau NFTs in positron emission tomography scans was consistently observed only in the presence of Aβ (Pontecorvo et al., 2017). However, a defined interaction between Aβ1–42 and Tau and their relative contribution to AD remains a controversial topic of research. For example, a recent in vivo study proposed that Tau is not required

for Aβ1–42-induced memory deficits (Puzzo et al., 2020). Untangling the neurobiological and clinical relationship between Aβ1–42 and Tau remains a necessary research objective.

In summary, the aetiology of AD is remarkably complex. The pathology encompasses a broad range of factors, including, but not limited to, genetic risk factors and molecular and cellular biochemical changes (Knopman et al., 2021).

3 | ADVANTAGES OF THE POWERFUL *DROSOPHILA MELANOGASTER* MODELLING SYSTEM: PRACTICALITY AND GENETICS

Genetic and epidemiological studies have provided a number of candidate pathways involved in disease (Karch & Goate, 2015); however, proving causality and identifying good targets for therapeutic intervention is crucial. This is where model organisms, such as *Drosophila*, are of paramount importance. Precise genetic engineering in *Drosophila* allows the targeting and isolation of specific genes. This enables an observation of causality between a genetic variant and the resulting phenotype.

Drosophila harbour an orthologue of APP, known as APP-like protein (APP-L) (Rosen et al., 1989), and have orthologues for all the γ-secretase components but have a single presenilin and APH gene (Prüßing et al., 2013). *Drosophila* strains have been generated expressing variant genes associated with familial AD (Fossgreen et al., 1998; Ye & Fortini, 1999) including APP, presenilin and other γ-secretase components (Bourdet et al., 2015; Chung & Struhl, 2001; Niimura et al., 2005). APOE is not conserved in *Drosophila* but Haddadi et al. (2016) created the first transgenic human APOE *Drosophila* model to investigate related neurodegenerative disease pathology. Despite harbouring orthologues of APP (APP-L), β-secretase and γ-secretase (Iijima et al., 2004; Prüßing et al., 2013; Takasugi et al., 2003), flies do not produce endogenous Aβ1–42 because the sequence corresponding to the Aβ1–42 peptide in APP-L lacks essential homology to the human region (Prüßing et al., 2013). Additionally, the β-secretase-like enzyme has been demonstrated to display low APP-L proteolytic activity (Fossgreen et al., 1998; Iijima et al., 2004). Nevertheless, over the last two decades, *Drosophila* has proven to be a valuable tool for studying amyloid proteotoxicity in AD.

Pathological processes in neurodegenerative disease often initiate decades before symptomatic onset which severely limits the use of human post-mortem tissue in investigating the full disease course (Lu & Vogel, 2009). Brain imaging studies on individuals carrying the autosomal dominant variants that result in early-onset familial AD have reported that Aβ accumulation occurs 15 years before the estimated age of symptomatic onset (Benzinger et al., 2013; Sperling et al., 2014). In contrast, disease progression in *Drosophila* models can be studied from initiation to the terminal stages because of their relatively short lifespan of 70–100 days (Piper & Partridge, 2018), and this is especially useful in late onset disease, such as AD. A primary advantage of *Drosophila* research is the ability to generate with relative ease large populations for widescale experiments, enabling statistical analysis. Females lay roughly 50 eggs per day at peak fecundity (Novoseltsev et al., 2003), and the generation time from a fertilised egg to an enclosed adult is approximately 10 days when reared at 25°C (Fernández-Moreno et al., 2007). This is another advantage over rodent models in which small sample sizes can provide statistical challenges. This provides a model that is highly sensitive in detecting modifiable changes in lifespan. *Drosophila* can also be fed a well-defined diet of sugar, yeast and agar (Piper & Partridge, 2018) in which pharmacological agents can be mixed into and thus facilitate easy administration of drugs to the flies.

However, arguably, the ease of genetic engineering in *Drosophila* is its major draw. The entire genome of the *Drosophila melanogaster* was sequenced in 2000 (Bergkvist et al., 2020) and is a powerful genetic tool kit to study human diseases and pathogenic mechanisms. *Drosophila* is very genetically tractable, and the evolution of sophisticated genome engineering techniques has facilitated intricate and precise genetic modification to be achievable. Thousands of *Drosophila* stocks, carrying variants, overexpression or RNAi constructs, can be easily acquired for a low cost from stock centres and as invertebrates, the use of *Drosophila* in medical research does not require regulatory licences or oversight (Piper & Partridge, 2018). *Drosophila* exhibits low genetic redundancy, which allows to uncover gene function more easily, and carry orthologues of around 75% of disease-causing genes in humans (Verheyen, 2022). Expression of human disease-associated genes in fly neurons usually results in deleterious phenotypes, analogous to those in humans. This makes flies an ideal model to uncover the function of disease-associated genes.

Fly disease models take advantage of the GAL4-UAS system of expression (Figure 2a), or the modified GS system (Figure 2b) which enables spatial and temporal control of transgene expression in *Drosophila* (Osterwalder et al., 1993). Recently, the developments of novel expression systems, such as the QF-QUAS and LexA-opA systems (Figure 2c,d), which can be combined with the GAL4-UAS system, allow the manipulation of genes in two tissues at the same time (Del Valle Rodríguez et al., 2012). This, for example, allows us to look at the interaction between Aβ in neurons and other genetic manipulations in glia, which will be invaluable as the field is increasingly appreciating

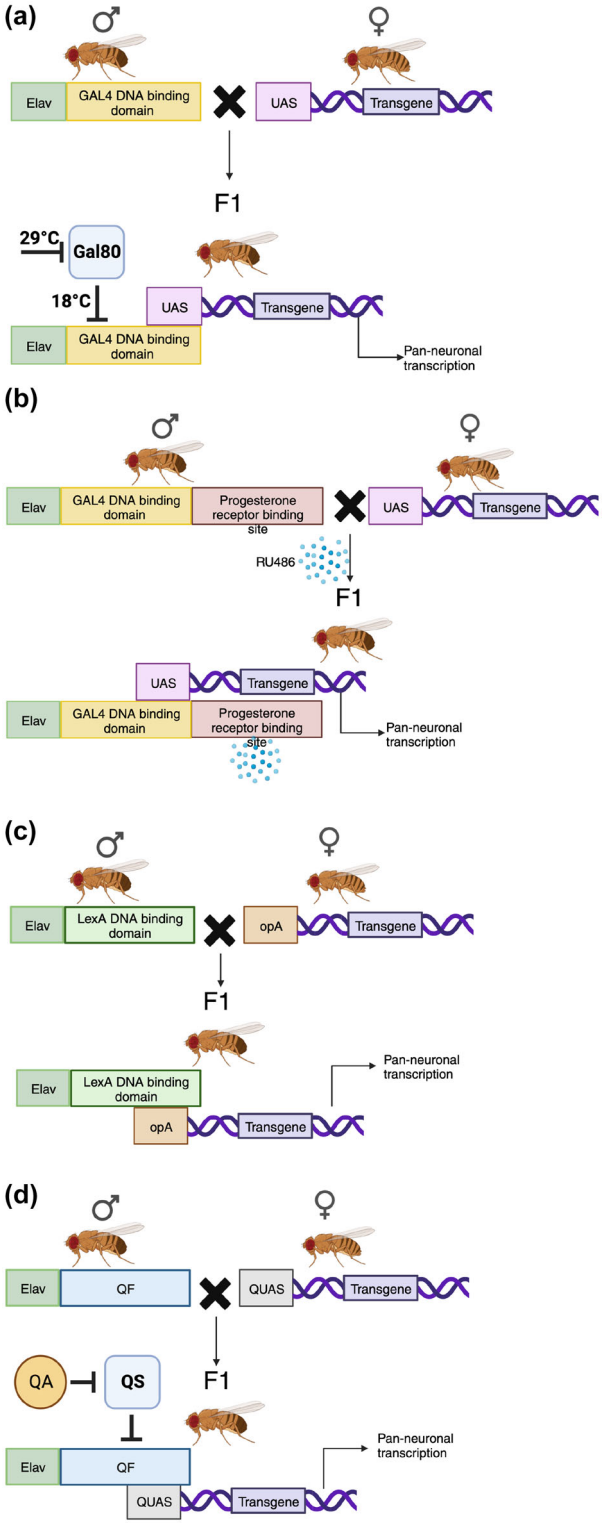

FIGURE 2 *Drosophila* transgene expression systems. (a) The UAS-GAL4 system. A GAL4 driver is cloned downstream of a tissue-specific promoter, leading to restricted production of the driver to the tissue of interest. The gene of interest is cloned downstream of an upstream activating sequence (UAS) promoter. When a fly carries both the GAL4 and UAS constructs, GAL4 is produced, it binds to its target UAS in the F1 offspring which drives transgene expression. At 18°C, GAL80 forms a complex with GAL4 to repress transgene transcription. GAL80-mediated transcription repression can be removed by exposing flies to 29°C. (b) The GS UAS-GAL4 system. GS GAL4 is a chimeric protein consisting of a GAL4 fused to a tissue specific promoter and the transcriptional activation domain of a progesterone receptor. Equivalent to the GAL4/UAS system, mating between a fly carrying GS GAL4 and a fly carrying a UAS-linked transgene of interest enables GAL4 to bind to its target UAS in the F1 offspring. Dissimilarly, transgene activation is only induced when the drug RU486 (mifepristone) binds to the progesterone receptor component, thus allowing temporal control via desired timing of RU486 application which can readily be applied to fly food. (c) The LexA-opA system. The LexA-opA system consists of the DNA binding domain of the transcription factor LexA, derived from *Escherichia coli*, and its target DNA sequences known as LexA operator sites (LexAop). LexA driver lines consist of the LexA transcription factor bound to a tissue-specific promoter to enable spatial control of the transgene. LexA driver line is mated with a line carrying a LexAop sequence fused upstream of the transgene of interest enabling LexA-LexAop binding which drives transgene expression tissue specifically. (d) The *Q* system. Similarly, mating between a fly carrying the transcriptional activator QF and a fly carrying transgene linked to a QUAS promoter enables QF to bind to its target QUAS in the F1 offspring which drives transgene expression. The repressor QS can bind to QF and inhibit activation of transgene expression. In the presence of the drug QA, QS-mediated repression of QF is inhibited. DNA, deoxyribonucleic acid; Elav, embryonic lethal abnormal vision; F1, filial 1; GS, gene-switch; QA, quinic acid; UAS, upstream activating sequence.

the importance of interactions among different cell types to disease development (Jonson et al., 2018).

4 | *DROSOPHILA* MODELS AS A TOOL FOR SCREENING

The facile genetics and the ease of carrying out experiments at scale, mean large-scale forward genetic screens can be used to rapidly identify disease modifiers in *Drosophila* models (Ugur et al., 2016). Typically, screens involve high throughput analysis of a plethora of variant *Drosophila* stocks, which are investigated for alterations in a pre-defined phenotype (Lenz et al., 2013); in the case of neurodegeneration, there are a number of established methodologies for assessing phenotypes (Figure 3).

4.1 | Eye morphology

The most commonly screened phenotype is the morphology of the fly eye. The *Drosophila* compound eye is a remarkable biological structure comprising around 750

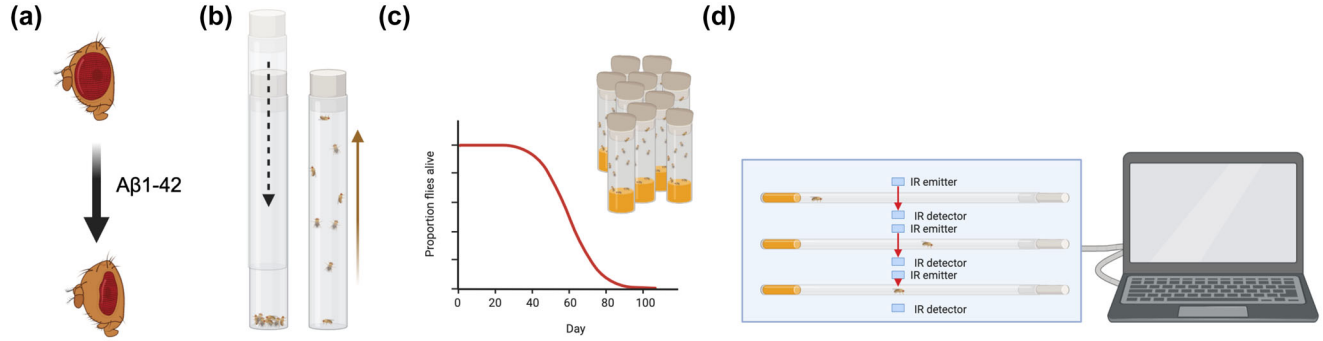

FIGURE 3 Established methodologies for assessing neurodegenerative phenotypes in *Drosophila*. (a) Assessment of eye morphology. A decrease in eye size and a rough eye appearance are morphological phenotypes that are widely accepted as a qualitative assessment of retinal neurotoxicity and neurodegeneration. GMR driver lines can be used to drive transgenic expression, for example, hAβ1–42 peptides, in the differentiating retinal photoreceptor neurons of the developing eye. (b) The climbing assay. Neuronal dysfunction and degeneration result in reduced locomotor behaviour in *Drosophila*, which can be observed as a reduction in climbing in the climbing assay. Flies are placed in a vial, mechanical stimulation via tapping the flies to the bottom of vial induces negative geotaxis, leading to a 'startle-response' of rapid climbing up the vial that is severely impaired in neurodegenerative phenotypes. (c) The lifespan assay. Modifiers of longevity can be easily assessed by scoring lifespan and statistically analysing the survivorship curve. (d) The *Drosophila* activity monitor (DAM) assay. The DAM assay is an automated tool that can assess locomotor behaviour over a continuous period of time in the absence of locomotion induction via the negative geotaxis response. Individual flies in sealed tubes are held in an 'activity monitor' in which an infrared beam detects every cross of the tube midpoint. Changes in normal locomotor behaviour can be detected by summing up the number of crossings per fly over a continuous period of time. GMR, Glass Multimer Repeat; hAβ1–42, human amyloid-beta 1–42 peptide.

ommatidia, meticulously arranged with remarkable precision, requiring 2/3 of essential genes for their correct development (Thaker & Kankel, 1992). They are neuronal in origin and provide a visible and easily scored metric for rapid identification of cell abnormalities or death, making them particularly suitable for neurodegeneration research (Iyer et al., 2016), even though eye morphology is often scored according to a qualitative score, leading to variable interpretations and weak modifiers may induce subtle variations that are undetectable by the naked eye (Iyer et al., 2016). However, recent technological advancements have led to the development of machine learning tools that can not only quantitatively assess the degeneration of the *Drosophila* eye but also categorise images into morphological classes using a pre-trained algorithm (Diez-Hermano et al., 2020), allowing for an unbiased quantitative assessment of eye morphology. Eye screens are a powerful screening tool; however, the morphology is mostly defined during development. Most neurodegenerative diseases are late onset, and therefore, phenotypes associated with ageing are more pertinent.

4.2 | Lifespan assays

Lifespan assays, where the survival of 100–150 flies is scored, allow to monitor genetic modifiers of longevity. Lifespan assays are frequently employed to demonstrate genetic/pharmacological modifier mediated rescues of the shortened lifespans exhibited in *Drosophila* amyloid toxicity models (Catterson et al., 2021; Finelli et al., 2004; Niccoli et al., 2016). As previously mentioned, *Drosophila* generally exhibits a lifespan of 70–100 days, which is much shorter than mammalian model organisms that typically live up to 3–5 years (Gorbunova et al., 2008). This characteristic makes *Drosophila* an ideal model organism for conducting large-scale longevity studies within a relatively short timeframe.

4.3 | Locomotor assays

Neuronal dysfunction and degeneration significantly affect locomotor ability, and thus, locomotor behaviour can be used as a more specific indicator of neurodegeneration in *Drosophila*. The most common assay is the climbing assay, and this is based on the negative geotaxis response, where flies will rapidly climb up the vial when tapped to the bottom of a vial (Chakraborty et al., 2011). Traditionally, climbing performance is measured as height achieved after a set time (Gargano et al., 2005), scored manually, recently the development of FreeClimber (Spierer et al., 2021), an open-source Python-based system allows the simultaneous monitoring and scoring of climbing speed of a population of flies, vastly speeding up the assay. *Drosophila* activity monitor is another automated system for locomotor activity that produces continuous data collection throughout a set period of time

(Pfeiffenberger et al., 2010). An 'activity monitor' holds individual flies in sealed tubes in which an infrared beam detects every cross of the tube midpoint. The number of crossings per fly can be summed up over a continuous period of time and reveal changes in normal locomotor activity (Pfeiffenberger et al., 2010). These tools are efficient computational methodologies that facilitate standardised and accurate analysis in addition to alleviating inherent systematic biases associated with conventional manual measurements. The significance of such tools is highly valuable in the context of modifier screens, as they enable rapid and reliable analysis of substantial volumes of data.

4.4 | Histological assays

It is widely accepted that the presence of vacuoles in the *Drosophila* brain reflects neuronal loss (Cook et al., 2012; Da Cruz et al., 2008; Loewen & Ganetzky, 2018). Quantification of the number and size of vacuoles in the *Drosophila* brain is a conventional method to assess the degree of neurodegeneration (Behnke et al., 2021; Herman et al., 1971; Ulian-Benitez et al., 2022) and is commonly employed in studies utilising *Drosophila* models of amyloid toxicity (Coelho et al., 2018; Iijima et al., 2008; Ray et al., 2017).

5 | *DROSOPHILA* MODELS OF AMYLOID TOXICITY

The two most widely employed methods to study amyloid toxicity in *Drosophila* are based on expressing directly the human Aβ1–42 peptide or co-expression of human APP (*hAPP*) with human β-secretase (*hBACE*) genes in *Drosophila* neurons. *Drosophila* has low endogenous proteolytic activity of β-secretase-like enzyme (Fossgreen et al., 1998) but the combination and expression of *hBACE*, with the assistance of endogenous *Drosophila* γ-secretase, facilitate the cleavage of *hAPP* into Aβ1–42 peptides which subsequently form Aβ plaques (Chakraborty et al., 2011; Greeve et al., 2004). *hAPP/hBACE* transgenic flies exhibit reduced lifespan compared to control wild-type flies (Chakraborty et al., 2011; Greeve et al., 2004), along with locomotor defects, as demonstrated by reduced climbing ability in the climbing assay (Chakraborty et al., 2011). When expression is driven in retinal photoreceptor neurons *hAPP/hBACE* co-expression leads to pronounced retinal neurodegeneration (Greeve et al., 2004). Transgenic *hAPP/hBACE* flies also have severe synaptic and neuroanatomical defects (Chakraborty et al., 2011; Mhatre et al., 2014), such as affecting mushroom bodies, which play a crucial role in learning and memory in the *Drosophila* brain (Chakraborty et al., 2011). The combined *hAPP/BACE* models allow for the correct processing of the APP protein. However, the majority of *Drosophila* models are based on direct expression of human Aβ1–42 (hAβ1–42) peptides in *Drosophila* neurons, and in order to guarantee its secretion, the hAβ1–42 peptide gene is fused to a signal peptide gene, typically necrotic (*Drosophila*) (Crowther et al., 2005), argos (*Drosophila*) (Casas-Tinto et al., 2011) or rat-proenkephalin (Finelli et al., 2004). Flies overexpressing hAβ1–42 exhibit significantly shorter lifespan, reduced locomotion, and retinal neurodegeneration (Crowther et al., 2005; Cutler et al., 2015; Finelli et al., 2004; Iijima et al., 2008; Niccoli et al., 2016). However, phenotypes are relatively mild when flies are carrying only one copy of hAβ1–42. Flies either homozygous for the Aβ peptide (Burnouf et al., 2015) or carrying bicistronic constructs, containing two tandem copies of hAβ1–42 (Casas-Tinto et al., 2011), display stronger lifespan and neurodegenerative phenotypes (Burnouf et al., 2015; Jeon et al., 2017; Xu et al., 2023). Flies expressing Aβ1–42 with an arctic variant, which is known to be associated with autosomal dominant AD (Balamurugan et al., 2017) and increased aggregation (Lu et al., 2019), display more severe deficits in locomotion and reduced longevity (Crowther et al., 2005; Iijima et al., 2008; Niccoli et al., 2016).

As well as models of Aβ1–42 toxicity, flies have been used to prove toxicity of novel Aβ species identified in human brains. For example, Aβ1–43 was shown for the first time in flies to trigger aggregation of Aβ1–40, which is usually not toxic (Burnouf et al., 2015), and pyroglutamate-modified Aβ ($A\beta_{pE3-42}$), an *N*-terminal modified Aβ species, was shown to be more toxic than Aβ1–42 itself (Sofola-Adesakin et al., 2016).

In summary, a number of fly models of AD have been developed to explore amyloid toxicity in *Drosophila*, some of the more common ones are displayed in Table 2. To note, this is not an exhaustive list of the models for amyloid toxicity that have been created. It can be considered a representation of the variety of models available.

6 | INSIGHTS FROM *DROSOPHILA* MODELS OF AMYLOID TOXICITY

Drosophila models offer valuable insights into establishing the causal effects of variants identified through human GWAS studies in influencing disease outcomes or uncovering potential downstream effectors of these variants. For instance, PICALM, a well-characterised risk factor for AD, has been demonstrated to affect synaptic glutamatergic signalling in an Aβ fly model (Yu et al., 2020). Similarly,

TABLE 2 The most common *Drosophila* models of amyloid toxicity.

Model	Description	System	Key phenotypic features	Reference
hAPP + *hBACE*	Transgenic lines co-expressing *hAPP* and *hBACE*	UAS-GAL4	Formation of Aβ plaques, accelerated neurodegeneration, synaptic abnormalities and shortened lifespan	(Greeve et al., 2004)
hAPP + *hBACE* + DPs variants	Additional co-expression of DPsn with point mutations corresponding to the familial AD pathogenic variants N141I, L235P and E280A	UAS-GAL4	Expression of pathogenic variants of the *Drosophila* presenilin gene accelerated Aβ-induced neurotoxicity	(Greeve et al., 2004)
hAβ1–42 peptide +arctic (necrotic SP)	Transgenic line expressing hAβ1–42 with/without arctic variant fused to the necrotic SP.	UAS-GAL4	The arctic variant results in increased neurotoxicity and Aβ protofibril formation	(Crowther et al., 2005)
hAβ1–42 peptide + arctic (ratPP SP)	Transgenic line expressing hAβ1–42 with/without arctic variant fused to the ratPP SP	UAS-GAL4	The arctic variant results in increased Aβ aggregation proneness, correlated to more severe deficits in memory/locomotor ability and shortened lifespan	(Iijima et al., 2008)
hAβ1–42 × 2 (argos SP)	Transgenic line expressing a construct with 2 tandem copies of hAβ1–42 fused to the argos SP	UAS-GAL4	2 independent Aβ1–42 copies induced extremely high levels of Aβ-induced neurotoxicity	(Casas-Tinto et al., 2011)
QUAS-Aβ42Arc	Transgenic line expressing hAβ1–42 with arctic variant	QF-QUAS	The arctic variant driven by the *Q* system reduced lifespan	(Niccoli et al., 2016)

Note: Models of amyloid toxicity in *Drosophila*.

Abbreviations: Aβ, amyloid beta; DPsn, *Drosophila* presenilin; hAPP, human amyloid precursor protein; hBACE, β-site amyloid precursor protein cleaving enzyme; ratPP, rat pre-proenkephalin; SP, signal peptide; UAS, upstream activating sequence.

TREM2, a potent disease modifier in humans, has been found to influence Tau toxicity but not amyloid toxicity in fly models. This observation suggests a potential specific role for TREM2 variants in the development of AD (Sekiya et al., 2018).

Furthermore, *Drosophila* models of amyloid toxicity enable researchers to delve into the fundamental neurodegenerative processes at the molecular and genetic levels. They help identify novel connections or expand upon findings from human studies, ultimately enhancing our understanding of disease mechanisms. For example, *Drosophila* models have demonstrated that the upregulation of glial engulfment receptors can rescue Aβ accumulation and toxicity, emphasising the significant role of glial cells in Aβ clearance (Ray et al., 2017). Additionally, the modulation of mitochondrial dynamics, achieved by overexpressing the regulator Miro, has been shown to mitigate neurodegeneration in AD fly models, suggesting a direct involvement of mitochondria in disease progression (Panchal & Tiwari, 2020).

Fly models have also helped to shed light on other crucial aspects of AD pathogenesis, stemming from observations of patients' symptoms. For instance, fly models have been used to show that the upregulation of glucose import in neurons can ameliorate amyloid toxicity, indicating that the drop in glucose metabolism observed in patient brains is a direct driver of disease (Niccoli et al., 2016). Similarly, considering the impact of gut microbiota dysbiosis on brain inflammation in humans, *Drosophila* Aβ models have revealed that enterobacteria infection exacerbates the progression of AD (Wu et al., 2017). *Drosophila* has also been employed to explore emerging research areas related to amyloid toxicity, offering insights that can potentially lead to the identification of novel therapeutic targets. For example, a recent paper demonstrated that the retention of proteins in the endoplasmic reticulum rescued amyloid toxicity in a *Drosophila* model but interestingly in the absence of diminishing Aβ expression levels (Catterson et al., 2021).

Drosophila models of amyloid toxicity have also been used to identify and screen potential therapeutic targets. *Drosophila* has been employed to investigate the impact of approved acetylcholine esterase inhibitors (aChEIs), such as rivastigmine, on amyloid toxicity and recently synthesised aChEIs (Siddique & Naz, 2022; Uras et al., 2021).

Arguably, the major advantage of *Drosophila* disease models lies within their ability to easily undergo large-scale screens for genetic modifiers, and this has been successfully applied to amyloid toxicity. The most commonly screened phenotype is the eye morphology, as this provides a quick, easily scorable and reliable method for measuring neurodegeneration. Cao et al. (2008) screened 1963 strains for modifiers of the Aβ1–42 model eye phenotype and found 23 modifiers, identifying proteostasis pathways and chromatin transcription regulation as important regulators of Aβ toxicity. Tan et al. (2008) using a similar screen, detected that loss of function of *Toll*, the *Drosophila* homolog of the mammalian Interleukin-1 (IL-1) receptor, strongly suppresses hAβ1–42-induced neuropathology. This finding implicates Aβ pathology in neuroinflammation, and following this study, inhibition of IL-1β, a pro-inflammatory cytokine, was demonstrated to prevent memory impairments in rodents (Williamson et al., 2011). Furthermore, treatment with fenamate non-steroidal anti-inflammatory drugs prevented memory deficits in an Aβ1–42 rodent model via inhibition of IL-1β secretion (Daniels et al., 2016). Screening via climbing and lifespan assays have also effectively identified candidate modifiers of Aβ1–42 neuropathology in *Drosophila* (Belfiori-Carrasco et al., 2017; Sanokawa-Akakura et al., 2010; Xu et al., 2023). Automated computational analysis of phenotypes is now becoming increasingly integrated into genetic screens, increasing the power and speed (Belfiori-Carrasco et al., 2017; Liu et al., 2015; Rival et al., 2009). Yang et al. (2023) used an automated image analysis tool to screen for naturally occurring variants as genetic modifiers of Aβ1–42 and Tau-induced eye phenotypes, identifying 14 unique variants, implicating neuronal and developmental processes in disease pathogenesis.

7 | LIMITATIONS OF *DROSOPHILA* MODELS OF AMYLOID TOXICITY

It is important to acknowledge that as an invertebrate species, a major drawback of *Drosophila* model systems are the substantial physiological and anatomical differences that limit translatability to humans. The *Drosophila* brain is considerably smaller in size with substantially fewer neurons in comparison to the human brain, which limits the capacity to accurately translate to the study of defined human brain regions, such as the hippocampus, and neural connectivity. Moreover, *Drosophila* lack an adaptive immune system (Padmanabhan & Götz, 2023), which limits their ability to accurately model neuroinflammation, a factor that is increasingly recognised as a significant contributor to AD pathogenesis (Uddin et al., 2021). Additionally, some common solvents for drug compounds are highly toxic to *Drosophila*, in particular dimethyl sulfoxide (Cvetković et al., 2015), which can interfere with pharmacological testing in *Drosophila* model systems. In contrast, they tolerate ethanol well up to 4%, as they naturally feed on rotting fruit with high ethanol concentrations; however, high concentrations of ethanol are also toxic (Bayliak et al., 2016). Therefore, it is good practice to incorporate vehicle-alone controls when performing drug-experiments.

Intriguingly, the direct expression of hAβ1–42 in fly neurons results in pronounced neurodegenerative phenotypes, despite the absence of plaques containing amyloid fibril structures (Iijima et al., 2004, 2008). Staining of the fly brain has revealed that hAβ1–42 overexpression presents as diffuse deposits (Iijima et al., 2004, 2008) which is in contrast to the *hAPP/hBACE* model where the formation of amyloid plaques with fibrillar structures is observable (Chakraborty et al., 2011; Greeve et al., 2004). Furthermore, it has been observed via staining that fly brains pan-neuronally expressing hAβ1–42 exhibit mainly intracellular localisation of amyloid deposits. There is a growing body of evidence in rodent models of amyloid toxicity that extracellular plaque deposition is preceded by intraneuronal Aβ1–42 accumulation (Billings et al., 2005; Wirths et al., 2001). Although this finding may diverge from the classical pathological hallmark of extracellular plaques composed of amyloid fibrils, it should not be perceived as a fundamental limitation. Instead, it indicates that the *Drosophila* model directly driving hAβ1–42 in neurons potentially reflects an earlier stage of amyloid toxicity.

It is crucial to acknowledge that *Drosophila* will never attain flawless recapitulation of an entire complex mammalian disease like AD. The principal advantage of *Drosophila* resides in their genetic manipulability, enabling the modelling of specific aspects of the disease, such as amyloid toxicity.

8 | CONCLUDING REMARKS

In AD research, *Drosophila* models offer quick, consistent and well-characterised phenotypes, allowing for high-throughput screening of gene and drug modifiers. While lacking mammalian features in the CNS, they remain invaluable for narrowing possibilities and pointing to new avenues that can then be further explored in mammalian and human systems.

AUTHOR CONTRIBUTIONS

Elli Tsintzas wrote the first draft, Teresa Niccoli reviewed and revised the article. All the authors read, reviewed, and approved the final version of the article.

ACKNOWLEDGMENTS

We thank our funders: ARUK Senior Fellowship ARUK-SRF2018A-003 (TN), an MRC grant MR/V003585/1 (TN), and a Dunhill Studentship (ET). We thank the associate editor and the reviewers for their useful feedback that improved this paper.

DATA AVAILABILITY STATEMENT

Data sharing not applicable, no new data generated.

ORCID

Teresa Niccoli https://orcid.org/0000-0001-9337-0411

REFERENCES

Alzheimer's Association. (2020). 2020 Alzheimer's disease facts and figures. [Alzheimer´s Association Report]. *Alzheimers Dementia*, *16*, 391–460.

Balamurugan, K., Murugan, N. A., Långström, B., Nordberg, A., & Ågren, H. (2017). Effect of Alzheimer familial chromosomal variants on the amyloid fibril interaction with different PET tracers: Insight from molecular modeling studies. *ACS Chemical Neuroscience*, *8*, 2655–2666.

Bales, K. R., Verina, T., Cummins, D. J., Du, Y., Dodel, R. C., Saura, J., Fishman, C. E., Delong, C. A., Piccardo, P., Petegnief, V., Ghetti, B., & Paul, S. M. (1999). Apolipoprotein E is essential for amyloid deposition in the APP(V717F) transgenic mouse model of Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 15233–15238.

Bayliak, M. M., Shmihel, H. V., Lylyk, M. P., Storey, K. B., & Lushchak, V. I. (2016). Alpha-ketoglutarate reduces ethanol toxicity in *Drosophila melanogaster* by enhancing alcohol dehydrogenase activity and antioxidant capacity. *Alcohol*, *55*, 23–33.

Behnke, J. A., Ye, C., Moberg, K. H., & Zheng, J. Q. (2021). A protocol to detect neurodegeneration in *Drosophila melanogaster* whole-brain mounts using advanced microscopy. *STAR Protocols*, *2*, 100689.

Bekris, L. M., Yu, C.-E., Bird, T. D., & Tsuang, D. W. (2010). Review article: Genetics of Alzheimer disease. *Journal of Geriatric Psychiatry and Neurology*, *23*, 213–227. https://doi.org/10.1177/0891988710383571

Belfiori-Carrasco, L. F., Marcora, M. S., Bocai, N. I., Ceriani, M. F., Morelli, L., & Castaño, E. M. (2017). A novel genetic screen identifies modifiers of age-dependent amyloid β toxicity in the *Drosophila* brain. *Frontiers in Aging Neuroscience*, *9*, 61.

Bellenguez, C., Küçükali, F., Jansen, I. E., Kleineidam, L., Moreno-Grau, S., Amin, N., Naj, A. C., Campos-Martin, R., Grenier-Boley, B., Andrade, V., Holmans, P. A., Boland, A., Damotte, V., Van Der Lee, S. J., Costa, M. R., Kuulasmaa, T., Yang, Q., De Rojas, I., Bis, J. C., … Lambert, J.-C. (2022). New insights into the genetic etiology of Alzheimer's disease and related dementias. *Nature Genetics*, *54*, 412–436.

Benzinger, T. L. S., Blazey, T., Jack, C. R., Koeppe, R. A., Su, Y., Xiong, C., Raichle, M. E., Snyder, A. Z., Ances, B. M., Bateman, R. J., Cairns, N. J., Fagan, A. M., Goate, A., Marcus, D. S., Aisen, P. S., Christensen, J. J., Ercole, L., Hornbeck, R. C., Farrar, A. M., … Morris, J. C. (2013). Regional variability of imaging biomarkers in autosomal dominant Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, E4502–E4509.

Bergkvist, L., Du, Z., Elovsson, G., Appelqvist, H., Itzhaki, L. S., Kumita, J. R., Kågedal, K., & Brorsson, A.-C. (2020). Mapping pathogenic processes contributing to neurodegeneration in *Drosophila* models of Alzheimer's disease. *FEBS Open Bio*, *10*, 338–350.

Billings, L. M., Oddo, S., Green, K. N., Mcgaugh, J. L., & Laferla, F. M. (2005). Intraneuronal Aβ causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. *Neuron*, *45*, 675–688.

Bloom, G. S. (2014). Amyloid-β and tau: The trigger and bullet in Alzheimer disease pathogenesis. *JAMA Neurology*, *71*, 505.

Bourdet, I., Preat, T., & Goguel, V. (2015). The full-length form of the *Drosophila* amyloid precursor protein is involved in memory formation. *Journal of Neuroscience*, *35*, 1043–1051.

Braak, H., & Braak, E. (1991). Neuropathological staging of Alzheimer-related changes. *Acta Neuropathologica*, *82*, 239–259.

Braak, H., & Braak, E. (1992). The human entorhinal cortex: Normal morphology and lamina-specific pathology in various diseases. *Neuroscience Research*, *15*, 6–31. https://doi.org/10.1016/0168-0102(92)90014-4

Budd Haeberlein, S., Aisen, P. S., Barkhof, F., Chalkias, S., Chen, T., Cohen, S., Dent, G., Hansson, O., Harrison, K., von Hehn, C., Iwatsubo, T., Mallinckrodt, C., Mummery, C. J., Muralidharan, K. K., Nestorov, I., Nisenbaum, L., Rajagovindan, R., Skordos, L., Tian, Y., … Sandrock, A. (2022). Two randomized phase 3 studies of aducanumab in early Alzheimer's disease. *Journal of Prevention of Alzheimer's Disease*, *9*, 197–210.

Burnouf, S., Gorsky, M. K., Dols, J., Grönke, S., & Partridge, L. (2015). Aβ43 is neurotoxic and primes aggregation of Aβ40 in vivo. *Acta Neuropathologica*, *130*, 35–47.

Caillé, I., Allinquant, B., Dupont, E., Bouillot, C., Langer, A., MüLler, U., & Prochiantz, A. (2004). Soluble form of amyloid precursor protein regulates proliferation of progenitors in the adult subventricular zone. *Development (Cambridge, England)*, *131*, 2173–2181.

Cao, W., Song, H.-J., Gangi, T., Kelkar, A., Antani, I., Garza, D., & Konsolaki, M. (2008). Identification of novel genes that modify phenotypes induced by Alzheimer's β-amyloid overexpression in *Drosophila*. *Genetics*, *178*, 1457–1471.

Casas-Tinto, S., Zhang, Y., Sanchez-Garcia, J., Gomez-Velazquez, M., Rincon-Limas, D. E., & Fernandez-Funez, P. (2011). The ER stress factor XBP1s prevents amyloid-β neurotoxicity. *Human Molecular Genetics*, *20*, 2144–2160.

Castellano, J. M., Kim, J., Stewart, F. R., Jiang, H., Demattos, R. B., Patterson, B. W., Fagan, A. M., Morris, J. C., Mawuenyega, K. G., Cruchaga, C., Goate, A. M., Bales, K. R., Paul, S. M., Bateman, R. J., & Holtzman, D. M. (2011). Human apoE isoforms differentially regulate brain amyloid-β peptide clearance. *Science Translational Medicine*, *3*, 89ra57.

Catterson, J. H. (2021) *Aβ toxicity rescued by protein retention in the ER*. bioRxiv. [Preprint] https://doi.org/10.1101/2021.08.18.456775

Cavazzoni, P. (2021). *FDA's decision to approve new treatment for Alzheimer's disease*. US Food and Drug Administration.

Chakraborty, R., Vepuri, V., Mhatre, S. D., Paddock, B. E., Miller, S., Michelson, S. J., Delvadia, R., Desai, A., Vinokur, M., Melicharek, D. J., Utreja, S., Khandelwal, P., Ansaloni, S., Goldstein, L. E., Moir, R. D., Lee, J. C., Tabb, L. P., Saunders, A. J., & Marenda,

D. R. (2011). Characterization of a *Drosophila* Alzheimer's disease model: Pharmacological rescue of cognitive defects. *PLoS ONE*, *6*, e20799.

Chapuis, J., Hansmannel, F., Gistelinck, M., Mounier, A., Van Cauwenberghe, C., Kolen, K. V., Geller, F., Sottejeau, Y., Harold, D., Dourlen, P., Grenier-Boley, B., Kamatani, Y., Delepine, B., Demiautte, F., Zelenika, D., Zommer, N., Hamdane, M., Bellenguez, C., Dartigues, J.-F., … Lambert, J.-C. (2013). Increased expression of BIN1 mediates Alzheimer genetic risk by modulating tau pathology. *Molecular Psychiatry*, *18*, 1225–1234.

Chen, G.-F., Xu, T.-H., Yan, Y., Zhou, Y.-R., Jiang, Y., Melcher, K., & Xu, H. E. (2017). Amyloid beta: Structure, biology and structure-based therapeutic development. *Acta Pharmacologica Sinica*, *38*, 1205–1235. https://doi.org/10.1038/aps.2017.28

Cheng, I. H., Palop, J. J., Esposito, L. A., Bien-Ly, N., Yan, F., & Mucke, L. (2004). Aggressive amyloidosis in mice expressing human amyloid peptides with the arctic variant. *Nature Medicine*, *10*, 1190–1192.

Chung, H.-M., & Struhl, G. (2001). Nicastrin is required for presenilin-mediated transmembrane cleavage in *Drosophila*. *Nature Cell Biology*, *3*, 1129–1132.

Coelho, D. S., Schwartz, S., Merino, M. M., Hauert, B., Topfel, B., Tieche, C., Rhiner, C., & Moreno, E. (2018). Culling less fit neurons protects against amyloid-β-induced brain damage and cognitive and motor decline. *Cell Reports*, *25*, 3661–3673.e3.

Cook, M., Mani, P., Wentzell, J. S., & Kretzschmar, D. (2012). RhoA prenylation in the loechrig (loe) variant leads to progressive neurodegeneration. *PLoS ONE*, *7*, e44440.

Crowther, D. C., Kinghorn, K. J., Miranda, E., Page, R., Curry, J. A., Duthie, F. A. I., Gubb, D. C., & Lomas, D. A. (2005). Intraneuronal Aβ, non-amyloid aggregates and neurodegeneration in a *Drosophila* model of Alzheimer's disease. *Neuroscience*, *132*, 123–135.

Cutler, T., Sarkar, A., Moran, M., Steffensmeier, A., Puli, O. R., Mancini, G., Tare, M., Gogia, N., & Singh, A. (2015). *Drosophila* eye model to study neuroprotective role of CREB binding protein (CBP) in Alzheimer's disease. *PLoS ONE*, *10*, e0137691.

Cvetković, V. J., Mitrovic, T. L., Jovanovic, B., Stamenkovic, S. S., Todorovic, M., Dordevic, M., & Radulovic, N. (2015). Toxicity of dimethyl sulfoxide against *Drosophila melanogaster*. *Biologica Nyssana*, *6*(2), 91–95.

Da Cruz, A. B., Wentzell, J., & Kretzschmar, D. (2008). Swiss cheese, a protein involved in progressive neurodegeneration, acts as a non-canonical regulatory subunit for PKA-C3. *Journal of Neuroscience*, *28*, 10885–10892.

Daniels, M. J. D., Rivers-Auty, J., Schilling, T., Spencer, N. G., Watremez, W., Fasolino, V., Booth, S. J., White, C. S., Baldwin, A. G., Freeman, S., Wong, R., Latta, C., Yu, S., Jackson, J., Fischer, N., Koziel, V., Pillot, T., Bagnall, J., Allan, S. M., … Brough, D. (2016). Fenamate NSAIDs inhibit the NLRP3 inflammasome and protect against Alzheimer's disease in rodent models. *Nature Communications*, *7*, 12504.

Office for National Statistics (ONS), released 15 December 2023, ONS website, statistical bulletin. Deaths registered in England and Wales. (2022). https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/bulletins/deathsregistrationsummarytables/2022#:~:text=In&2022%2C&there&were&577%2C160,last&the&case&in&1981

Del Valle Rodríguez, A., Didiano, D., & Desplan, C. (2012). Power tools for gene expression and clonal analysis in *Drosophila*. *Nature Methods*, *9*, 47–55. https://doi.org/10.1038/nmeth.1800

De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Guhde, G., Annaert, W., Von Figura, K., & Van Leuven, F. (1998). Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature*, *391*(6665), 387–390.

Dib, S., Pahnke, J., & Gosselet, F. (2021). Role of abca7 in human health and in Alzheimer's disease. *International Journal of Molecular Sciences*, *22*, 4603. https://doi.org/10.3390/ijms22094603

Diez-Hermano, S., Ganfornina, M. D., Vegas-Lozano, E., & Sanchez, D. (2020). Machine learning representation of loss of eye regularity in a *Drosophila* neurodegenerative model. *Frontiers in Neuroscience*, *14*, 516.

Fernández-Moreno, M. A., Farr, C. L., Kaguni, L. S., & Garesse, R. (2007). *Drosophila melanogaster* as a model system to study mitochondrial biology. *Methods in Molecular Biology*, *372*, 33–49.

Finelli, A., Kelkar, A., Song, H.-J., Yang, H., & Konsolaki, M. (2004). A model for studying Alzheimer's Aβ42-induced toxicity in *Drosophila melanogaster*. *Molecular and Cellular Neuroscience*, *26*, 365–375.

Fossgreen, A., Brückner, B., Czech, C., Masters, C. L., Beyreuther, K., & Paro, R. (1998). Transgenic *Drosophila* expressing human amyloid precursor protein show γ-secretase activity and a blistered-wing phenotype. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 13703–13708.

Gargano, J., Martin, I., Bhandari, P., & Grotewiel, M. (2005). Rapid iterative negative geotaxis (RING): A new method for assessing age-related locomotor decline in *Drosophila*. *Experimental Gerontology*, *40*, 386–395.

Gorbunova, V., Bozzella, M. J., & Seluanov, A. (2008). Rodents for comparative aging studies: From mice to beavers. *Age*, *30*, 111–119. https://doi.org/10.1007/s11357-008-9053-4

Greeve, I., Kretzschmar, D., Tschäpe, J.-A., Beyn, A., Brellinger, C., Schweizer, M., Nitsch, R. M., & Reifegerste, R. (2004). Age-dependent neurodegeneration and Alzheimer-amyloid plaque formation in transgenic *Drosophila*. *Journal of Neuroscience*, *24*, 3899–3906. https://doi.org/10.1523/JNEUROSCI.0283-04.2004

Grudzien, A., Shaw, P., Weintraub, S., Bigio, E., Mash, D. C., & Mesulam, M. M. (2007). Locus coeruleus neurofibrillary degeneration in aging, mild cognitive impairment and early Alzheimer's disease. *Neurobiology of Aging*, *28*, 327–335.

Haass, C., & Willem, M. (2019). Secreted APP modulates synaptic activity: A novel target for therapeutic intervention? *Neuron*, *101*, 557–559. https://doi.org/10.1016/j.neuron.2019.01.058

Haddadi, M., Nongthomba, U., Jahromi, S. R., & Ramesh, S. R. (2016). Transgenic *Drosophila* model to study apolipoprotein E4-induced neurodegeneration. *Behavioural Brain Research*, *301*, 10–18.

Herman, M. M., Miquel, J., & Johnson, M. (1971). Insect brain as a model for the study of aging. *Acta Neuropathologica*, *19*, 167–183.

Husain, M. A., Laurent, B., & Plourde, M. (2021). APOE and Alzheimer's disease: From lipid transport to physiopathology and therapeutics. *Frontiers in Neuroscience*, *15*, 630502. https://doi.org/10.3389/fnins.2021.630502

Iijima, K., Chiang, H.-C., Hearn, S. A., Hakker, I., Gatt, A., Shenton, C., Granger, L., Leung, A., Iijima-Ando, K., & Zhong, Y. (2008).

Aβ42 variants with different aggregation profiles induce distinct pathologies in *Drosophila*. *PLoS ONE*, *3*, e1703.

Iijima, K., Liu, H.-P., Chiang, A.-S., Hearn, S. A., Konsolaki, M., & Zhong, Y. (2004). Dissecting the pathological effects of human Aβ40 and Aβ42 in *Drosophila*: A potential model for Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 6623–6628.

Iyer, J., Wang, Q., Le, T., Pizzo, L., Grönke, S., Ambegaokar, S. S., Imai, Y., Srivastava, A., Troisí, B. L., Mardon, G., Artero, R., Jackson, G. R., Isaacs, A. M., Partridge, L., Lu, B., Kumar, J. P., & Girirajan, S. (2016). Quantitative assessment of eye phenotypes for functional genetic studies using *Drosophila melanogaster*. *G3: Genes, Genomes, Genetics*, *6*, 1427–1437.

Jeon, Y., Lee, S., Shin, M., Lee, J. H., Suh, Y. S., Hwang, S., Yun, H. S., & Cho, K. S. (2017). Phenotypic differences between *Drosophila* Alzheimer's disease models expressing human Aβ42 in the developing eye and brain. *Animal Cells and Systems*, *21*, 160–168.

Jonson, M., Nyström, S., Sandberg, A., Carlback, M., Michno, W., Hanrieder, J., Starkenberg, A., Nilsson, K. P. R., Thor, S., & Hammarström, P. (2018). Aggregated Aβ1–42 is selectively toxic for neurons, whereas glial cells produce mature fibrils with low toxicity in *Drosophila*. *Cell Chemical Biology*, *25*, 595–610. e5.

Karch, C. M., & Goate, A. M. (2015). Alzheimer's disease risk genes and mechanisms of disease pathogenesis. *Biological Psychiatry*, *77*, 43–51. https://doi.org/10.1016/j.biopsych.2014.05.006

Karran, E., & De Strooper, B. (2022). The amyloid hypothesis in Alzheimer disease: New insights from new therapeutics. *Nature Reviews Drug Discovery*, *21*, 306–318. https://doi.org/10.1038/s41573-022-00391-w

Khera, R., & Das, N. (2009). Complement receptor 1: Disease associations and therapeutic implications. *Molecular Immunology*, *46*, 761–772. https://doi.org/10.1016/j.molimm.2008.09.026

Kimberly, W. T., Lavoie, M. J., Ostaszewski, B. L., Ye, W., Wolfe, M. S., & Selkoe, D. J. (2003). γ-Secretase is a membrane protein complex comprised of presenilin, nicastrin, aph-1, and pen-2. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 6382–6387.

Knopman, D. S., Amieva, H., Petersen, R. C., Chételat, G., Holtzman, D. M., Hyman, B. T., Nixon, R. A., & Jones, D. T. (2021). Alzheimer disease. *Nature Reviews Disease Primers*, *7*, 33.

Kondo, J., Honda, T., Mori, H., Hamada, Y., Miura, R., Ogawara, M., & Ihara, Y. (1988). The carboxyl third of tau is tightly bound to paired helical filaments. *Neuron*, *1*, 827–834.

Kumar-Singh, S., Theuns, J., Van Broeck, B., Pirici, D., Vennekens, K., Corsmit, E., Cruts, M., Dermaut, B., Wang, R., & Van Broeckhoven, C. (2006). Mean age-of-onset of familial Alzheimer disease caused by presenilin variants correlates with both increased Aβ42 and decreased Aβ40. *Human Mutation*, *27*, 686–695.

Lemere, C. A., Blusztajn, J. K., Yamaguchi, H., Wisniewski, T., Saido, T. C., & Selkoe, D. J. (1996). Sequence of deposition of heterogeneous amyloid β-peptides and APO E in down syndrome: Implications for initial events in amyloid plaque formation. *Neurobiology of Disease*, *3*, 16–32.

Lenz, S., Karsten, P., Schulz, J. B., & Voigt, A. (2013). *Drosophila* as a screening tool to study human neurodegenerative diseases. *Journal of Neurochemistry*, *127*, 453–460. https://doi.org/10.1111/jnc.12446

Leroy, K., Ando, K., Laporte, V., Dedecker, R., Suain, V., Authelet, M., Héraud, C., Pierrot, N., Yilmaz, Z., Octave, J.-N., & Brion, J.-P. (2012). Lack of tau proteins rescues neuronal cell death and decreases amyloidogenic processing of APP in APP/PS1 mice. *American Journal of Pathology*, *181*, 1928–1940.

Lewis, J., Dickson, D. W., Lin, W. L., Chisholm, L., Corral, A., Jones, G., Yen, S. H., Sahara, N., Skipper, L., Yager, D., Eckman, C., Hardy, J., Hutton, M., & McGowan, E. (2001). Enhanced neurofibrillary degeneration in transgenic mice expressing variant tau and APP. *Science (1979)*, *293*, 1487–1491.

Li, N. M. (2019). Variants of beta-amyloid precursor protein alter the consequence of Alzheimer's disease pathogenesis. *Neural Regeneration Research*, *14*, 658–665.

Liu, H., Han, M., Li, Q., Zhang, X., Wang, W. A., & Huang, F. D. (2015). Automated rapid iterative negative geotaxis assay and its use in a genetic screen for modifiers of Aβ42-induced locomotor decline in *Drosophila*. *Neuroscience Bulletin*, *31*, 541–549.

Loewen, C. A., & Ganetzky, B. (2018). Mito-nuclear interactions affecting lifespan and neurodegeneration in a *Drosophila* model of leigh syndrome. *Genetics*, *208*, 1535–1552.

Lu, B., & Vogel, H. (2009). *Drosophila* models of neurodegenerative diseases. *Annual Review of Pathology: Mechanisms of Disease*, *4*, 315–342. https://doi.org/10.1146/annurev.pathol.3.121806.151529

Lu, M., Williamson, N., Mishra, A., Michel, C. H., Kaminski, C. F., Tunnacliffe, A., & Kaminski Schierle, G. S. (2019). Structural progression of amyloid- arctic variant aggregation in cells revealed by multiparametric imaging. *Journal of Biological Chemistry*, *294*, 1478–1487.

Mhatre, S. D., Satyasi, V., Killen, M., Paddock, B. E., Moir, R. D., Saunders, A. J., & Marenda, D. R. (2014). Synaptic abnormalities in a *Drosophila* model of Alzheimer's disease. *DMM Disease Models and Mechanisms*, *7*, 373–385.

Mikol, J., Brion, S., Guicharnaud, L., & Waks, O. (2001). Formation of neurofibrillary tangles in P301L tau transgenic mice induced by a Abeta 42 fibrils. *Science (1979)*, *293*, 1491–1495.

Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winblad, B., & Lannfelt, L. (1992). A pathogenic variant for probable Alzheimer's disease in the APP gene at the *N*–terminus of β–amyloid. *Nature Genetics*, *1*, 345–347.

Nandi, A., Counts, N., Chen, S., Seligman, B., Tortorice, D., Vigo, D., & Bloom, D. E. (2022). Global and regional projections of the economic burden of Alzheimer's disease and related dementias from 2019 to 2050: A value of statistical life approach. *eClinicalMedicine*, *51*, 101580.

Niccoli, T., Cabecinha, M., Tillmann, A., Kerr, F., Wong, C. T., Cardenes, D., Vincent, A. J., Bettedi, L., Li, L., Grönke, S., Dols, J., & Partridge, L. (2016). Increased glucose transport into neurons rescues Aβ toxicity in *Drosophila*. *Current Biology*, *26*, 2291–2300.

Nichols, E., Steinmetz, J. D., Vollset, S. E., Fukutaki, K., Chalek, J., Abd-Allah, F., Abdoli, A., Abualhasan, A., Abu-Gharbieh, E., Akram, T. T., Al Hamad, H., Alahdab, F., Alanezi, F. M., Alipour, V., Almustanyir, S., Amu, H., Ansari, I., Arabloo, J., Ashraf, T., … Vos, T. (2022). Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: An analysis for the Global Burden of Disease Study 2019. *Lancet Public Health*, *7*, e105–e125.

Niimura, M., Isoo, N., Takasugi, N., Tsuruoka, M., Ui-Tei, K., Saigo, K., Morohashi, Y., Tomita, T., & Iwatsubo, T. (2005). Aph-1 contributes to the stabilization and trafficking of the γ-secretase complex through mechanisms involving intermolecular and intramolecular interactions. *Journal of Biological Chemistry*, *280*, 12967–12975.

Nilsberth, C., Westlind-Danielsson, A., Eckman, C. B., Condron, M. M., Axelman, K., Forsell, C., Stenh, C., Luthman, J., Teplow, D. B., Younkin, S. G., Näslund, J., & Lannfelt, L. (2001). The 'arctic' APP variant (E693G) causes Alzheimer's disease by enhanced Aβ protofibril formation. *Nature Neuroscience*, *4*, 887–893.

Novoseltsev, V. N., Novoseltseva, J. A., Boyko, S. I., & Yashin, A. I. (2003). What fecundity patterns indicate about aging and longevity: Insights from *Drosophila* studies. *Journals of Gerontology—Series A Biological Sciences and Medical Sciences*, *58*, B484–B494.

Osterwalder, T., Yoon, K. S., White, B. H., Keshishian, H., & O'malley, B. W. (1993). A conditional tissue-specific transgene expression system using inducible GAL4. *PNAS*, *98*(22), 12596–12601. www.pnas.org

Padmanabhan, P., & Götz, J. (2023). Clinical relevance of animal models in aging-related dementia research. *Nature Aging*, *3*, 481–493.

Panchal, K., & Tiwari, A. K. (2020). Miro, a Rho GTPase genetically interacts with Alzheimer's disease-associated genes (Tau, Aβ42 and Appl) in *Drosophila melanogaster*. *Biology Open*, *9*, bio049569.

Pfeiffenberger, C., Lear, B. C., Keegan, K. P., & Allada, R. (2010). Locomotor activity level monitoring using the *Drosophila* activity monitoring (DAM) system. *Cold Spring Harbor Protocols*, *2010*, Nov 1;2010(11):pdb.prot5518.

Piper, M. D. W., & Partridge, L. (2018). *Drosophila* as a model for ageing. *Biochimica et Biophysica Acta—Molecular Basis of Disease*, *1864*, 2707–2717. https://doi.org/10.1016/j.bbadis.2017.09.016

Pontecorvo, M. J., Sr Devous, M. D., Navitsky, M., Lu, M., Salloway, S., Schaerf, F. W., Jennings, D., Arora, A. K., McGeehan, A., Lim, N. C., Xiong, H., Joshi, A. D., Siderowf, A., Mintun, M. A., & 18F-AV-1451-A05 investigators. (2017). Relationships between flortaucipir PET tau binding and amyloid burden, clinical diagnosis, age and cognition. *Brain*, *140*, 748–763.

Prüßing, K., Voigt, A., & Schulz, J. B. (2013). *Drosophila melanogaster* as a model organism for Alzheimer's disease. *Molecular Neurodegeneration*, *8*, 35. http://www.molecularneurodegeneration.com/content/8/1/35

Puzzo, D., Argyrousi, E. K., Staniszewski, A., Zhang, H., Calcagno, E., Zuccarello, E., Acquarone, E., Fa', M., Li Puma, D. D., Grassi, C., D'adamio, L., Kanaan, N. M., Fraser, P. E., & Arancio, O. (2020). Tau is not necessary for amyloid-ß-induced synaptic and memory impairments. *Journal of Clinical Investigation*, *130*, 4831–4844.

Quartey, M. O., Nyarko, J. N. K., Maley, J. M., Barnes, J. R., Bolanos, M. A. C., Heistad, R. M., Knudsen, K. J., Pennington, P. R., Buttigieg, J., De Carvalho, C. E., Leary, S. C., Parsons, M. P., & Mousseau, D. D. (2021). The Aβ(1–38) peptide is a negative regulator of the Aβ(1–42) peptide implicated in Alzheimer disease progression. *Scientific Reports*, *11*, 431.

Ray, A., Speese, S. D., & Logan, M. A. (2017). Glial draper rescues Aβ toxicity in a *Drosophila* model of Alzheimer's disease. *Journal of Neuroscience*, *37*, 11881–11893.

Rival, T., Page, R. M., Chandraratna, D. S., Sendall, T. J., Ryder, E., Liu, B., Lewis, H., Rosahl, T., Hider, R., Camargo, L. M., Shearman, M. S., Crowther, D. C., & Lomas, D. A. (2009). Fenton chemistry and oxidative stress mediate the toxicity of the -amyloid peptide in a *Drosophila* model of Alzheimer's disease. *European Journal of Neuroscience*, *29*, 1335–1347.

Roberson, E. D., Scearce-Levie, K., Palop, J. J., Yan, F., Cheng, I. H., Wu, T., Gerstein, H., Yu, G. Q., & Mucke, L. (2007). Reducing endogenous tau ameliorates amyloid β-induced deficits in an Alzheimer's disease mouse model. *Science (1979)*, *316*, 750–754.

Rodríguez-Rivera, C., Garcia, M. M., Molina-Álvarez, M., González-Martín, C., & Goicoechea, C. (2021). Clusterin: Always protecting. Synthesis, function and potential issues. *Biomedicine and Pharmacotherapy*, *134*, 111174. https://doi.org/10.1016/j.biopha.2020.111174

Rosen, D. R., Martin-Morris, L., Luo, L. Q., & White, K. (1989). A *Drosophila* gene encoding a protein resembling the human β-amyloid protein precursor. *Proceedings of the National Academy of Sciences of the United States of America*, *86*, 2478–2482.

Rosenberg, R. N., Lambracht-Washington, D., Yu, G., & Xia, W. (2016). Genomics of Alzheimer disease: A review. *JAMA Neurology*, *73*, 867. https://doi.org/10.1001/jamaneurol.2016.0301

Sanokawa-Akakura, R., Cao, W., Allan, K., Patel, K., Ganesh, A., Heiman, G., Burke, R., Kemp, F. W., Bogden, J. D., Camakaris, J., Birge, R. B., & Konsolaki, M. (2010). Control of Alzheimer's amyloid beta toxicity by the high molecular weight immunophilin FKBP52 and copper homeostasis in *Drosophila*. *PLoS ONE*, *5*, e8626.

Sekiya, M., Wang, M., Fujisaki, N., Sakakibara, Y., Quan, X., Ehrlich, M. E., De Jager, P. L., Bennett, D. A., Schadt, E. E., Gandy, S., Ando, K., Zhang, B., & Iijima, K. M. (2018). Integrated biology approach reveals molecular and pathological interactions among Alzheimer's Aβ42, Tau, TREM2, and TYROBP in *Drosophila* models. *Genome Medicine*, *10*, 26.

Siddique, Y. H., Naz, F., Varshney, R., & Varshney, H. (2022). Comparative study of rivastigmine and galantamine on the transgenic *Drosophila* model of Alzheimer's disease. *Current Research in Pharmacology and Drug Discovery*, *3*, 100120.

Sofola-Adesakin, O., Khericha, M., Snoeren, I., Tsuda, L., & Partridge, L. (2016). pGluAβ increases accumulation of Aβ in vivo and exacerbates its toxicity. *Acta Neuropathologica Communications*, *4*, 109.

Sperling, R., Mormino, E., & Johnson, K. (2014). The evolution of preclinical Alzheimer's disease: Implications for prevention trials. *Neuron*, *84*, 608–622. https://doi.org/10.1016/j.neuron.2014.10.038

Spierer, A. N., Yoon, D., Zhu, C. T., & Rand, D. M. (2021). FreeClimber: Automated quantification of climbing performance in *Drosophila*. *Journal of Experimental Biology*, *224*, jeb229377.

Steiner, H., Duff, K., Capell, A., Romig, H., Grim, M. G., Lincoln, S., Hardy, J., Yu, X., Picciano, M., Fechteler, K., Citron, M., Kopan, R., Pesold, B., Keck, S., Baader, M., Tomita, T., Iwatsubo, T., Baumeister, R., & Haass, C. (1999). A loss of function variant of presenilin-2 interferes with amyloid β- peptide production and Notch signaling. *Journal of Biological Chemistry*, *274*, 28669–28673.

Swanson, C. J., Zhang, Y., Dhadda, S., Wang, J., Kaplow, J., Lai, R. Y. K., Lannfelt, L., Bradley, H., Rabe, M., Koyama, A., Reyderman, L., Berry, D. A., Berry, S., Gordon, R., Kramer, L. D., & Cummings, J. L. (2022). Correction to: A randomized, double-blind, phase 2b proof-of-concept clinical trial in early Alzheimer's disease with lecanemab, an anti-Aβ protofibril antibody. *Alzheimer's Research and Therapy*, *14*, 70. https://doi.org/10.1186/s13195-022-00995-9

Takasugi, N., Tomita, T., Hayashi, I., Tsuruoka, M., Niimura, M., Takahashi, Y., Thinakaran, G., & Iwatsubo, T. (2003). The role of presenilin cofactors in the gamma-secretase complex. *Nature*, *422*, 438–441.

Tan, L., Schedl, P., Song, H.-J., Garza, D., & Konsolaki, M. (2008). The Toll→NFκB signaling pathway mediates the neuropathological effects of the human Alzheimer's Aβ42 polypeptide in *Drosophila*. *PLoS ONE*, *3*, e3966.

Tebar, F., Bohlander, S. K., & Sorkin, A. (1999). Clathrin assembly lymphoid myeloid leukemia (CALM) protein: Localization in endocytic-coated pits, interactions with clathrin, and the impact of overexpression on clathrin-mediated traffic. *Molecular Biology of the Cell*, *10*, 2687–2702.

Thaker, H. M., & Kankel, D. R. (1992). Mosaic analysis gives an estimate of the extent of genomic involvement in the development of the visual system in *Drosophila melanogaster*. *Genetics*, *131*, 883–894.

Theofilas, P., Ehrenberg, A. J., Dunlop, S., Di Lorenzo Alho, A. T., Nguy, A., Leite, R. E. P., Rodriguez, R. D., Mejia, M. B., Suemoto, C. K., Ferretti-Rebustini, R. E. D. L., Polichiso, L., Nascimento, C. F., Seeley, W. W., Nitrini, R., Pasqualucci, C. A., Jacob Filho, W., Rueb, U., Neuhaus, J., Heinsen, H., … Grinberg, L. T. (2017). Locus coeruleus volume and cell population changes during Alzheimer's disease progression: A stereological study in human postmortem brains with potential implication for early-stage biomarker discovery. *Alzheimer's and Dementia*, *13*, 236–246.

U.S. Food and Drug Administration (FDA). (2021). *FDA grants accelerated approval for Alzheimer's drug*. FDA News Release.

Uddin, M. S., Kabir, M. T., Jalouli, M., Rahman, M. A., Jeandet, P., Behl, T., Alexiou, A., Albadrani, G. M., Abdel-Daim, M. M., Perveen, A., & Ashraf, G. M. (2021). Neuroinflammatory signaling in the pathogenesis of Alzheimer's disease. *Current Neuropharmacology*, *20*, 126–146.

Ugur, B., Chen, K., & Bellen, H. J. (2016). *Drosophila* tools and assays for the study of human diseases. *Disease Models and Mechanisms*, *9*, 235–244. https://doi.org/10.1242/dmm.023762

Ulian-Benitez, S., Hjelmstad, A. S., Barbosa, G. O., & Haghighi, P. (2022). Eosin whole-brain mount staining to analyze neurodegeneration in a fly model of Alzheimer's disease. *STAR Protocols*, *3*, 101377.

Ulland, T. K., & Colonna, M. (2018). TREM2 — A key player in microglial biology and Alzheimer disease. *Nature Reviews Neurology*, *14*, 667–675. https://doi.org/10.1038/s41582-018-0072-1

Uras, G., Manca, A., Zhang, P., Markus, Z., Mack, N., Allen, S., Bo, M., Xu, S., Xu, J., Georgiou, M., & Zhu, Z. (2021). In vivo evaluation of a newly synthesized acetylcholinesterase inhibitor in a transgenic *Drosophila* model of Alzheimer's disease. *Frontiers in Neuroscience*, *15*, 691222.

Van Der Lee, S. J., Wolters, F. J., Ikram, M. K., Hofman, A., Ikram, M. A., Amin, N., & Van Duijn, C. M. (2018). The effect of APOE and other common genetic variants on the onset of Alzheimer's disease and dementia: A community-based cohort study. *Lancet Neurology*, *17*, 434–444.

van Dyck, C. H. (2022). Lecanemab in early Alzheimer's disease. *New England Journal of Medicine*, *388*(1), 9–21.

Van Hoesen, G. W., Hyman, B. T., & Damasio, A. R. (1991). Entorhinal cortex pathology in Alzheimer's disease. *Hippocampus*, *1*, 1–8. https://doi.org/10.1002/hipo.450010102

Verheyen, E. M. (2022). The power of *Drosophila* in modeling human disease mechanisms. *Disease Models and Mechanisms*, *15*, dmm049549. https://doi.org/10.1242/DMM.049549

Williamson, L. L., Sholar, P. W., Mistry, R. S., Smith, S. H., & Bilbo, S. D. (2011). Microglia and memory: Modulation by early-life infection. *Journal of Neuroscience*, *31*, 15511–15521.

Wirths, O., Multhaup, G., Czech, C., Blanchard, V., Moussaoui, S., Tremp, G., Pradier, L., Beyreuther, K., & Bayer, T. A. (2001). Intraneuronal Aβ accumulation precedes plaque formation in β-amyloid precursor protein and presenilin-1 double-transgenic mice. *Neuroscience Letters*, *306*, 116–120.

Wu, S.-C., Cao, Z.-S., Chang, K.-M., & Juang, J.-L. (2017). Intestinal microbial dysbiosis aggravates the progression of Alzheimer's disease in *Drosophila*. *Nature Communications*, *8*, 24.

Xu, D., Vincent, A., González-Gutiérrez, A., Aleyakpo, B., Anoar, S., Giblin, A., Atilano, M. L., Adams, M., Shen, D., Thoeng, A., Tsintzas, E., Maeland, M., Isaacs, A. M., Sierralta, J., & Niccoli, T. (2023). A monocarboxylate transporter rescues frontotemporal dementia and Alzheimer's disease models. *PLoS Genetics*, *19*, e1010893.

Yang, M., Zinkgraf, M., Fitzgerald-Cook, C., Harrison, B. R., Putzier, A., Promislow, D. E. L., & Wang, A. M. (2023). Using *Drosophila* to identify naturally occurring genetic modifiers of amyloid beta 42- and tau-induced toxicity. *G3: Genes, Genomes, Genetics*, *13*, jkad132.

Ye, Y., & Fortini, M. E. (1999). Apoptotic activities of wild-type and Alzheimer's disease-related variant presenilins in *Drosophila melanogaster*. *Journal of Cell Biology*, *146*, 1351–1364.

Yu, Y., Niccoli, T., Ren, Z., Woodling, N. S., Aleyakpo, B., Szabadkai, G., & Partridge, L. (2020). PICALM rescues glutamatergic neurotransmission, behavioural function and survival in a *Drosophila* model of Aβ42 toxicity. *Human Molecular Genetics*, *29*, 2420–2434.

How to cite this article: Tsintzas, E., & Niccoli, T. (2024). Using *Drosophila* amyloid toxicity models to study Alzheimer's disease. *Annals of Human Genetics*, 1–15. https://doi.org/10.1111/ahg.12554