Review Article

Drosophila melanogaster: a veritable genetic tool and *in vivo* model for human Alzheimer's disease

ABSTRACT

The rise in the cases of neurodegenerative diseases, such as the familial forms of Alzheimer's disease is worrisome and a burden to many societies in our ever-increasing world. Due to the complexity in the nature of the brain and spinal cord characterized by an extremely organized network of neuronal cells, there is a need to answer scientific inquiries in uncomplicated, though similar, systems. *Drosophila melanogaster* (fruit-fly) is a well-studied and easily managed genetic model organism used for discerning the molecular mechanisms of many human diseases. There are strong conservations of several basic biological, physiological and neurological features between *D. melanogaster* and mammals, as about 75% of all human disease-causing genes are considered to possess a functional homolog in the fruit-fly. The development of *Drosophila* models of several neurodegenerative disorders via developed transgenic technologies has presented spectacular similarities to human diseases. An advantage that the fruit-fly has over other model organisms, such as the mouse, is its comparatively brief lifespan, which allows complex inquiries about brain functions to be addressed more quickly. Furthermore, there have been steady increases in understanding the pathophysiological basis of many neurological disorders via genetic screenings with the aid of *Drosophila* models. This review presents a widespread summary of the fruit-fly models relevant to Alzheimer's disease, and highlight important genetic modifiers that have been recognized using this model.

Keywords: Drosophila melanogaster, Alzheimer's disease, neurodegeneration, amyloids, tauopathies

INTRODUCTION

Neurological diseases as explained by the World Health Organization (WHO), the World Bank, and the Harvard School of Public Health are among the largest burdens to global public health and warn that it might escalate to an uncontrollable global issue [1]. As a result of the aforementioned, numerous scientifically-oriented strategies are necessary to delineate the etiologies of diseases, their progression, and possible management; so as to help in comprehending diseases onsets and associated risk factors, likewise the framework of treatment and possible interventions. The ranges of diseases under the categories of neurological disorders are so wide and difficult, with more than 600 of such disorders reported by the National Institute of Neurological Disorders and Stroke [2]. They include neurodegenerative, neurodevelopmental, cancer, stroke and traumatic injuries.

One of the most efficient and outstanding ways to gain meaningful insight and knowledge of diseases is to conceptualize and design disease mechanisms and identify possible disease-modifying pathways and signals in similar, mini-complex organisms. The use of *Drosophila melanogaster* (*D. melanogaster*) widely known as the fruit-fly has produced lofty advancements with respect to the understanding of

several neurological and neurodegenerative diseases. The fruit-fly has not succeeded in illuminating the comprehension of many biological signals and pathways which are dysfunctional in disease conditions, but likewise the backbone needed for efficient modalities and intervention patterns in various mammalian organs and systems.

A good grasp of *Drosophila* genetics have also allowed the fruit-fly to be engineered into useful models for studying the pathophysiological basis and mechanisms underlying may neurological disorders ravaging humans. Also worthy of note, are the meaningful advances that have been recorded through the use of the fruit-fly in the study of memory, locomotion, learning, circadian rhythms, and other human-related neurobehaviors.

This review focuses on studies that have used targeted misexpression of human diseases-associated proteins to model Alzheimer's disease. Though, this work is not posed to be a comprehensive and exclusive outlook, due to the ever-increasing landscape of Alzheimer's disease; nevertheless, any reader with little or no knowledge in *Drosophila* and its genetics would acknowledge the impacts that fruit-flies models have contributed to the knowledge of neurodegenerative diseases.

DROSOPHILA MELANOGASTER AS A MODEL ORGANISM

History of *D. melanogaster*

The use of *D. melanogaster* in biological sciences date as far back as a century ago, and the rich history of its use and applications cannot be exclusively captured in this review. Since its introduction over 100 years, the fruit-fly has gained prominence as a veritable tool employed to understand genes, chromosome and the inheritance of genetic information [3]. One of the notable scientific feats which were first discovered from the use of fly was that heritable traits are located on the chromosomes, amongst other ground-breaking records in genetics.

A glossary looks at the recipient of the prestigious Nobel Prize for Physiology and Medicine in the year 1994, Ed Lewis was known for his outstanding work on gene structure using the fruit-flies models. Also worthy of note is the work of Eric Weischaus and Christiane Nusslein-Volhard who uncovered the various processes of embryogenesis responsible for the identification of several genes involved in all phases of development. A good number of these genes have been established to play a pivotal role in the development of mammalian systems.

In recent times, with regards to genome sequencing, *D. melanogaster* happens to be the first primary complex organism whose genome was sequenced [4]. A major highlight of this breakthrough was the striking similarities that exist between the homologs of humans and the fruit-fly, which in no small measure confirms the suitability of the fruit-fly as a remarkable model to study human biology and diseases mechanisms.

In years to come, the fruit-fly will remain at the core of biology and science, where significant discoveries are first conceptualized in the fruit-fly before been translated to other living systems.

Basic Biology of *D. melanogaster*

The complete sequencing and annotation of *D. melanogaster* genome have been successfully carried out and it currently encodes for over 14,000 genes located on

four chromosomes, of which the majority of the genome is found on three alone. There are confirmed reports that about 75% of disease-related genes in humans have functional orthologs in the fruit-fly [5].

D. melanogaster has a fast life cycle as compared to other organisms and models. For example, a fertile mating process could give rise to genetically similar offspring in their hundreds within 8 to 12 days at a favorable temperature of 25^{o}C. However, this is different from what is obtainable in rodents, who are only able to produce few offspring within a duration of 12 to 16 weeks. *D. melanogaster* model is regarded as multiple organisms due to its various stages of development: the embryo, larva, pupa, and adult, with each having its own uniqueness and distinct benefits.

The embryo of the fly is useful for studying the development of the fly, such as organogenesis, the formation of patterns, neuronal development, amongst others. The larva, with emphasis on the third instar larva, is employed to examine the physiological and development processes, alongside specific behaviors. The pupal phase is characterized with robust morphological transformations that produce the final adult fly; therefore the pupa serves as a good model to investigate specific processes of fly development. The adult fly is a complex organism with structures that carry out similar functions as seen in a mammalian heart, kidney, lung, gut, reproductive tract, amongst others. Its brain consists of over 100,000 neurons that form networks and circuits that regulate multiple behaviors, such as, sleep, memory, courtship, flight control, circadian rhythms, feeding, amongst others.

COMPARISON BETWEEN *D. MELANOGASTER* AND HUMANS

Similarities between *D. melanogaster* and Humans

An important speculation concerning the use of invertebrate models to understand neurodegenerative disorders is that considerable features underpinning the biology of flies and humans are preserved. It is, therefore, necessary to know how similar is the fruit-fly and humans. In general terms, there exist similarities between the fly and humans in the basic areas of cell biology, such as cell signaling, regulation of gene expression, synaptogenesis, neuronal connections, and cell death. Several genes and pathways that were initially discovered in fruit-flies have now been elucidated in mammals. A good example of such is the *Drosophila wingless* (Wnt) gene and pathway.

Differences between *D. melanogaster* and Humans

There are certain differences that exist between fruit-flies and humans. For instance, *D. melanogaster* possesses simple cognitive processes and circulatory systems. The simplistic genomic makeup of fly as compared to humans may be useful for genetic analysis. In fruit-flies, there is the absence of redundancy and possible duplication of genes as seen in humans. This advantage can help to break down the analysis of various biological processes in the fruit-fly. Furthermore, genetic manipulations which seem impossible in mammals are available using invertebrate models. Also within a short timeframe, fruit-flies can be reproduced in a large number, thereby making them readily available for screening which could lead to groundbreaking identification of rare mutations.

UNDERSTANDING NEURODEGENRATION USING GENETIC APPLICATIONS

It is widely reported that about 75% of the total genes involved in certain human diseases possess at least one homolog in *Drosophila melanogaster*. The comprehensive information of these fly homologs can be retrieved from an online source via http://superfly.ucsd.edu/homophila/. The homologs of genes for several neurodegenerative diseases in humans can be obtained in the fruit-fly genome. The study of the functions of respective genes can be carried out via generation of mutations in the fruit-fly homologs, after which the resultant phenotypes are subjected to further examinations. The use of this distinct approach has been employed to study numerous genes associated with neurodegenerative diseases. Notable among them are parkin, a gene related with autosomal recessive juvenile parkinsonism [6], [7]; ataxin-2, the gene mutated in spinocerebellar ataxia [8], and atrophin, a gene associated with dentatorubral pallidoluysian atrophy (DRPLA) [9]. Another powerful technique involves the use of RNA interference-mediated knockdown of gene expression, which was instrumental in delineating the pivotal function played by the fly homolog of Huntington's disease in apoptosis and axonal transport regulation [10] (Figure 1).

JUSTIFICATION FOR STUDYING NEURODEGENERATION IN *D. melanogaster*

It is possible to study in fruit-fly any pathogenic event of interest in humans, provided such processes can be reproduced with distinct features similar to what is seen in man. The use of genetic techniques can be utilized to delineate these pathogenic processes. The generation of mutations specific to certain pathogenic event can be employed to understand the mechanisms, signals, and pathways of diseases without having to make mere and unfound assumptions (Table 1). These outstanding prospects of using different genetic approaches and tools to delineate and uncover pathogenic processes and events further confirms the fruit-fly as a valuable, veritable and powerful model system in neurosciences.

THE *D. melanogaster* EYE AS A VERITABLE MODEL

The eye of *D. melanogaster* has been at the forefront and focus of fruit-fly research, since the year 1910, when a white-eyed fruit-fly was discovered in Morgan's lab at Columbia. The fruit-fly eyes are peculiar because the phenotypes of the adult eyes can be detected easily, it can tolerate genetic manipulation of some biological processes, and the eyes are dispensable for the survival of the flies. With the aid of the fruit-fly eyes, sophisticated techniques have been deployed to generate, detect and characterize certain mutations that have helped in the understanding of gene functions. Several studies have reported the use of fruit-fly eyes to extensively study various biological and physiological processes such as cell proliferation and differentiation, cell cycle regulation, neuronal circuitry, apoptosis, tissue formation, amongst others.

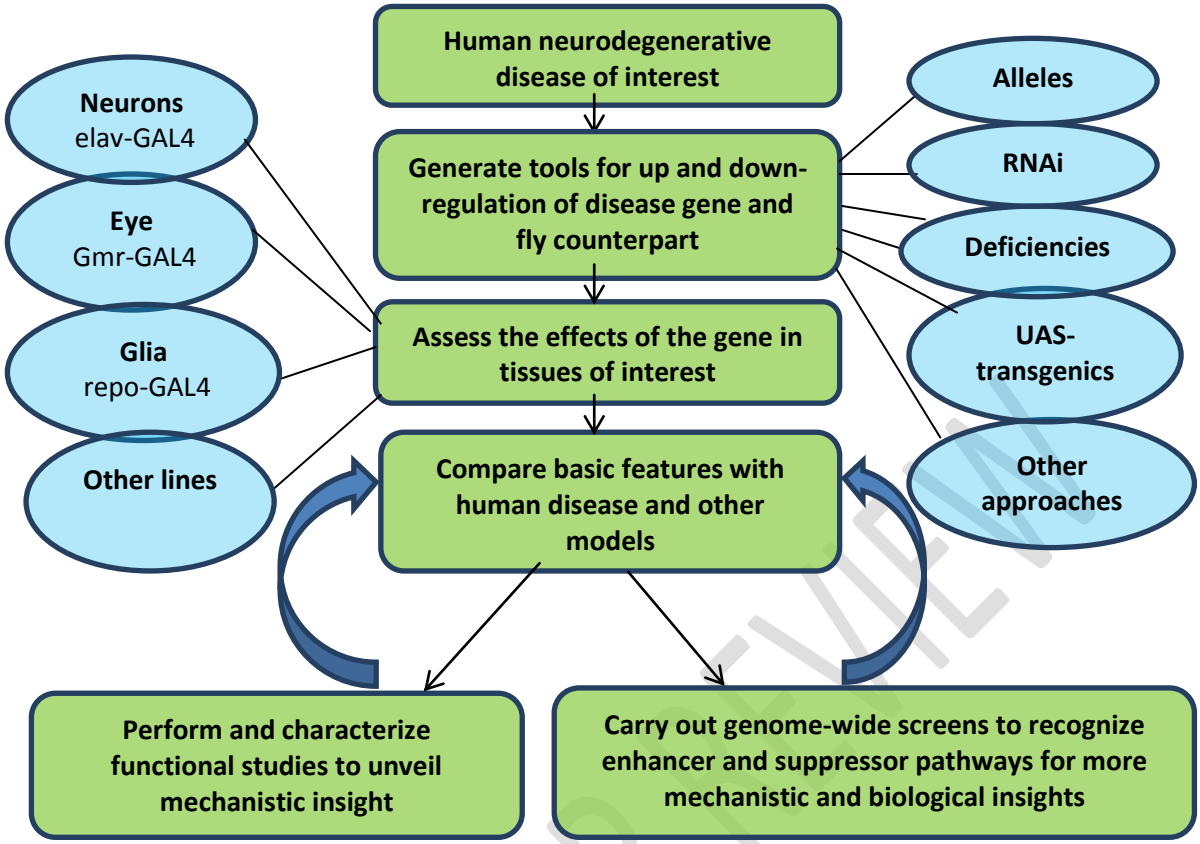

Figure 1: Phases involved in generating and characterizing a Drosophila melanogaster model for neurodegenerative disease in humans.

D. melanogaster AND ITS APPLICATION TO ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is regarded as the most common neurodegenerative disease. Its features include progressive dysfunctions in memory and cognition with a characteristic onset at the late age of life. The pathologic features of Alzheimer's disease are selective atrophy of the hippocampus and frontal cerebral cortex, and its hallmarks are amyloid plaques and neurofibrillary tangles (NFT).

Amyloid

Extracellular amyloid plaque is one of the significant neuropathological characteristics of Alzheimer's disease. Aβ peptide obtained from a membrane-bound amyloid precursor protein (APP) is a major component of these amyloid plaques [11]. Two distinct pathways are responsible for producing APP namely; the amyloidogenic pathway, which give rise to the production of Aβ, and the non-amyloidogenic pathway, which produces a secreted form of APP. An early-onset familial Alzheimer's disease can be caused by a dominant mutation in amyloid precursor protein (APP), or presenilins 1 and 2 [12], [13].

Interestingly, the homologs of both APP and preselinin are obtainable in *Drosophila*. Though the APP homolog found in the fruit-fly, *Appl*, lack the segment of APP required to produce pathogenic peptides; however, genetic applications has revealed the possible function of *Appl* in flies. Deletions in fly *Appl* gene presented defects in locomotive behavior, which was corrected by a human β-APP transgene [14]. A study by Toroja *et al.*, (1999) also suggested a possible role of fruit-fly *Appl* in synaptogenesis [15].

Lately, some research groups have presented fly models of AD via the use of misexpression of Aβ. One of such studies was performed by Iijima *et al*., [16], where a signal peptide obtained from pre-proenkephalin cleaved to Aβ was used to produce secreted transgene materials. The resulting production of the toxic peptide, Aβ42 brought about the development of diffuse extracellular amyloid, defected olfactory associative learning, and neurodegeneration in the fly models. A similar technique was used by Finelli *et al.,* (2004) [17], who observed its effects in the eyes of the fruit-flies, and was occasioned with a resultant retinal degeneration.

Also, the genetic screening and isolation of neprilysin 2 as a potential modifier that is capable of suppressing the Aβ42 phenotype when it is overexpressed has been successfully carried out [17]. Finding from a study showed the involvement of neprilysin in Aβ degradation [18]. A report from the findings of Greeve *et al*., [19], suggested the presence of retinal neurodegeneration and amyloid plaque-like formation in fruit-flies that co-express APP alongside with either β-secretase or a dominant-negative form of presenilin. The impairment of axonal transport by APP in mice, fruit-fly, and Alzheimer's disease brain has been investigated by Goldstein and Gunawardena [20], and Stokin *et al.,* [21].

β- and γ-secretase are accountable for the production of pathogenic Aβ peptides. Though the characterization of β-secretase has been achieved, the specific proteins liable for the activity of γ-secretase are unidentifiable [11]. The homolog of presenilin, which is considered to be one of the constituents of the γ-secretase complex, has been successfully characterized in the Drosophila model and is named *Psn*. *Psn* is needed for the regular proteolytic processes of *Notch,* and its mutations are able to produce phenotypes which remind us of the *Notch* mutants [22], [23].

The use of other invertebrates concepts via *Drosophila* genomics and *Caenorhabditis elegans* have been employed to discover other constituents of the γ-secretase complex [24], which includes Aph-1, Pen-2, and nicastrin. The homologs of all the constituents have been established in the fruit-fly, and have been confirmed to be capable of being a portion of the γ-secretase complex [25]. Another study conducted by Guo *et al*., [26], reported the identification of other elements of the γ-secretase complex via a genetic system using a GAL4-responsive rough eye phenotype.

Tauopathies

The development of neurofibrillary tangle (NFT) is another significant feature observed in the pathology of Alzheimer's disease. Nevertheless, neurofibrillary dysfunction is evident in other disorders jointly called tauopathies. They include corticobasal degeneration, fronto-temporal dementia, and progressive supranuclear palsy [27]. Tau can be described as a microtubule-associated protein, whose connection with microtubules is negatively controlled by phosphorylation of sites located in or around its microtubule-binding repeats.

Tauopathies are believed to be occasioned by the presence of abnormal control of tau phosphorylations which lead to microtubule-binding, and the hyperphosphorylation of tau is perceived to play a role in the conversion of tau proteins from soluble to insoluble forms. *Drosophila* tau homologs have been successfully copied and qualified, and tauopathy models have been replicated in fruit-fly models in few studies [28]. A study conducted by Williams and his colleagues [29] showed that the overexpression of human tau in sensory neurons developed a

number of aberrant morphologic outcomes, such as swelling and axonal degeneration and loss. Also, in a new study, these researchers reported that the impaired motor behavior and axonal transport defects made by tau was enhanced by the misexpression of an organically active form of the tau kinase glycogen synthase kinase (GSK)-3β [30].

Another related study carried out by Wittman and his team [31] produced an overexpression of the wild type, alongside the FTDP-17-associated mutants R406W and V337M mutant tau in the CNS of the fruit-fly. In this study, both the wild type and R406W tau resulted in vacuolization and neuronal loss; however, the observed pathology was intense with the mutant tau. In addition, the immunoreactivity for different epitopes of phosphotau tends to accumulate over time with no evidence of neurofibrillary abnormalities. Furthermore, when the above study [31] was expressed in the retina of *Drosophila*, a rough eye phenotype was discovered with R406W but not in wild-type tau, indicating that rough eye phenotype reduced the complexity associated with modifier screens.

In another study by Shulman and Feany, their findings showed that tau modifiers have been found from a genetic screen [32]. These modifiers comprise mainly of phosphatases and kinases, supporting the significance of phosphorylation of tau in its pathogenicity. Nevertheless, there has not been any report as to whether the modifiers caused any change in the solubility or phosphorylation of tau. Also, the ability of tau misexpression to alter olfactory learning and memory has been reported in a study [33], while another finding established the improvement of tau pathogenicity by coexpression with *Sgg*, and therefore proposed that phosphorylation by the kinase PAR-1 is necessary for further phosphorylation by other kinases like GSK-3 [34].

Table 1: List of *D. melanogaster* models for Alzheimer's disease

Serial number	Gene or protein	References
1	APP	[35], [36]
2	Aβ peptide	[16], [37]
3	PSEN 1 and 2	[38], [39]
4	MAPT (Tau)	[31], [40]

USE OF *D. melanogaster* IN DRUG DISCOVERY FOR ALZHEIMER'S DISEASE

It has been established that most of the genes involved in the pathogenesis of Alzheimer's disease (AD) have *D. melanogaster* homologs; for example, the homolog for human APP in fruit-fly is the APP-like or APPL. Several scientific findings have shown that fruit-flies that lack APPL present behavioral dysfunction that can be greatly subdued by the expression of human APP transgene, which is an indication of functional conservation between human APP and *Drosophila* APPL [41], though few differences exist.

Till present, there are limited published studies targeted to identify new potential drugs for treating AD using the *D. melanogaster* model system via screening processes. The scientific breakthrough recorded through the development of several invertebrate models, particularly the *D. melanogaster* models of AD, supplies superior tools for carrying out drug screens in order to identify potent molecules that

are capable of conquering the toxicity connected with Aβ aggregation and thereby regulate the activity of γ-secretase.

SUMMARY AND PERSPECTIVES

The prospects of the fruit-fly are high and will be sustained as an impressive and vital complementary model to unveil important biology, provided dynamic approaches and the constant addition of novel tools to control the fly genome are employed. The use of these state-of-the-art tools in conjunction with more polished techniques will help us to acknowledge the biology and gain a deeper molecular understanding of primary biological and physiological processes. In addition, it reveals how these processes are implicated in diseases, thereby unraveling the mysteries of brain function, its' possible reactions to aging, and the abnormal state. The use of *D. melanogaster* in research will keep on rendering necessary foundations needed for the evolution of therapeutics required to palliate several destructive diseases of the brain.

CONCLUSION

D. melanogaster has proven to be an extraordinary tool for rendering valuable understanding into many biological and physiological processes; here this paper emphasized how it has been employed for several targeted studies of neurodegeneration, especially Alzheimer's disease. As a veritable model, it reflects- with striking resemblance- neurodegenerative disease dysfunctions in mammals. This review stressed the strength of the fruit-fly and how it has been incorporated with mammalian/human studies and genetics, thereby giving rooms for a new line of understanding.

D. melanogaster is able to further supply functional aid in many ways for human molecular genetics studies with the use of sophisticated human genomic sequencing technologies. A good example is the use of genome-wide association studies (GWAS) to unravel modifiers that may impact the risk of disease in humans; yet, a dispute with GWAS is that the association domains are large and consists of several loci, making it so hard in identifying the specific gene involved in the disease process. Beside this, is the complexity in deciphering how the gene-related to disease-risk is connected with disease effect. However, the sustained dedication of *Drosophila* researchers and scientists to produce novel, electrifying applications, and approaches, combined with new breakthroughs into disease physiology, guarantees that the fruit-fly model will go on as an indispensable and veritable biological and physiological counterpart for studying a majority of human diseases.

CONSENT

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ETHICAL APPROVAL

I declare that this study have been examined and approved by the appropriate ethics committee and have therefore been performed following the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

The author declares that he has no competing interest.

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