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## Parkinson's Disease: Insights from *Drosophila* Model

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### Abstract

Parkinson's disease (PD) is a medical condition that has been known since ancient times. It is the second most common neurodegenerative disorder affecting approximately 1% of the population over 50 years. It is characterized by both motor and non-motor symptoms. Most of PD cases are sporadic while 5–10% cases are familial. Environment factors such as exposure to pesticides, herbicides and other heavy metals are expected to be the main cause of sporadic form of the disease. Mutation of the susceptible genes such as SNCA, PINK1, PARKIN, DJ1, and others are considered to be the main cause of the familial form of disease. *Drosophila* offers many advantages for studying human neurodegenerative diseases and their underlying molecular and cellular pathology. Shorter life span; large number of progeny; conserved molecular mechanism(s) among fly, mice and human; availability of many techniques, and tools to manipulate gene expression makes *drosophila* a potential model system to understand the pathology associated with PD and to unravel underlying molecular mechanism(s) responsible for dopaminergic neurodegeneration in PD—understanding of which will be of potential assistance to develop therapeutic strategies to PD. In the present review, we made an effort to discuss the contribution of fly model to understand pathophysiology of PD, in understanding the biological functions of genes implicated in PD; to understand the gene-environment interaction in PD; and validation of clues that are generated through genome-wide association studies (GWAS) in human through fly; further to screen and develop potential therapeutic molecules for PD. In nutshell, fly has been a great model system which has immensely contributed to the biomedical research relating to understand and addressing the pathology of human neurological diseases in general and PD in particular.

**Keywords:** dopamine, *Drosophila*, Parkinson's disease, mitochondrial dysfunction, neuroprotective therapeutics, pathophysiology and translational research

## 1. Introduction

Parkinson's disease (PD) is a medical condition that has been known about since ancient times in Indian and Chinese civilization [1, 2]. It is referred to as *Kampavata* in the ancient Indian medical system of Ayurveda ("*kampa*" means tremor in Sanskrit). An Egyptian papyrus from the twelfth century BC mentions a king drooling with age [3]. In Western medical literature, the tremor symptom was described by the physician Galen in 175 AD [4]. In 1817, James Parkinson wrote an essay on "shaking palsy" based on six cases that he had observed in his own practice and on walks around his neighborhood. The essay was intended to encourage others to study the disease. This established the disease as a recognized medical condition. He termed this medical condition as "*shaking palsy* or *paralysis agitans*". He published a detailed medical essay entitled "An Essay on the Shaking Palsy" where he described shaking palsy as "involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forwards, and to pass from a walking to a running pace: the senses and intellects being uninjured" [5]. The term "Parkinson's disease" was coined in 1865 by William Sanders and later popularized by French neurologist Jean Martin Charcot [6]. Charcot and colleagues described the clinical symptoms of this disease, noting two prototypes: the tremorous and the rigid or akinetic form. They described the detailed arthritic changes, dysautonomia, and pain that can accompany Parkinson's disease. He recognized that PD patients are not markedly weak and do not necessarily have tremor [7]. All these observations instigated the curiosity among the clinicians to understand this condition better with an aim to improve the patient's quality of life.

## 2. Pathophysiology

Parkinson's disease is the second most common neurodegenerative disorder affecting approximately 1% of the population over 50 years [8]. A central pathological hallmark of PD is the selective loss of dopamine (DA) neurons in the *substantia nigra pars compacta* (SN). These dopaminergic neurons are required for proper motor function, and their deficiency manifests its characteristic features: bradykinesia, tremors, and rigidity [9]. A second neuropathological hallmark of PD is the Lewy body (LB), which is a cytoplasmic spherical proteinaceous inclusion. LBs have been reported to contain various proteins including  $\alpha$ -synuclein, ubiquitin, parkin, and neurofilaments [10]. The mechanisms by which  $\alpha$ -synuclein and other proteins aggregate to form Lewy pathology are uncertain, but may involve oxidative modifications and/or cross-linking. Although, the neurodegeneration of PD was considered to be confined to dopaminergic cell loss in the SN, cell loss, and neuropathology is found to occur in other parts as well including the locus coeruleus, raphe, nucleus basalis of Meynert, dorsal motor nucleus of the vagus, cerebral cortex, olfactory bulb, and autonomic nervous system [11]. Several non-motor symptoms such as sleep disturbances, constipation, cognitive decline, depression, fear, anxiety, bladder problems, weight changes, fatigue and loss of energy, hypotension, and sexual problems can be dominant and debilitating in a sizeable number of patients, affecting the quality of their life [12]. Till date, treatments address only the symptoms but they fail to stop the progression of the disease and PD patients continue to experience a higher mortality rate compared to the general population [13].

## 2.1. Sporadic Parkinson's disease

A sporadic PD has unknown cause with implication of environmental influence coupled with genetic factors. The pathology of PD therefore may be multifactorial involving gene and environment interactions. Studies indicate role of neurotoxins or neuroprotective compounds in pathogenesis of nigrostriatal degeneration, supporting the concept of association between the environment and PD [14]. Additionally, the identification of the mutated  $\alpha$ -synuclein (SNCA) gene that cause familial PD [15] as a risk factor for sporadic disease [16] provides a genetic background for the disease. Studies suggest that rural people, well water consumption, pesticide use, and occupations like rural farming, mining, and welding have an increased risk of PD [17, 18]. Epidemiological studies suggest association of PD with environmental toxic factors, primarily the mitochondrial complex I inhibitors such as rotenone [19]. Some other findings suggest that exposure to pesticide such as bipyridyl, organochlorine, and carbamate derivatives could contribute to PD [20].

## 2.2. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

Exploring the contribution of environmental exposure markedly advanced our understanding of the mechanisms involved in the development of PD. Initial evidence came from findings that subjects exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) developed PD-like symptoms [21]. MPTP was accidentally discovered in a synthesis process that went wrong, and, although it may have caused some problems in certain circles, today it represents the most important and most frequently used parkinsonian toxin applied in animal models. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a representative strong neurotoxin that has been recognized from several young drug addicts from Northern California developed severe parkinsonism [22]. Since then, environmental exposure to pesticides [23], polychlorinated biphenyls [24], organic solvents [25], metals [26], and air pollutants [27] has been proposed to increase risk for PD.

## 2.3. Mechanism of MPTP neurotoxicity

Though the exact mechanism regarding the mode of MPTP toxicity is not known, it has been postulated that MPP<sup>+</sup> entry into dopaminergic neurons is dependent on selective uptake by dopamine transporter localizing it and interfering into mitochondrial activity. MPTP is not toxic per se, but becomes toxic once it is converted to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP<sup>+</sup>) by action of monoamine oxidase B (MAO-B) in glial cells and serotonergic neurons [28] followed by oxidation into 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>), which is a highly toxic compound [29]. Then the dopamine transporter (DAT) carries it to dopaminergic neurons leading to its accumulation in cytoplasm and into synaptic vesicles by the vesicular monoamine transporter (VMAT). The driving force of mitochondrial membrane potential lets MPP<sup>+</sup> enter these organelles, where it blocks complex I [30]. This leads to abnormally increased concentrations of the toxin to interfere with mitochondrial respiration by blocking the mitochondrial oxidation. Thus it results in impairment of ATP synthesis and involving in the generation of oxidative stress.

## 2.4. Paraquat

Paraquat (1,1-dimethyl-4,4'-bipyridinium dichloride) is a quaternary nitrogen herbicide widely used for broadleaf weed control. It is a fast-acting, non-selective compound which destroys tissues of green plants on contact and by translocation with the plant. Significant damage to the brain was seen in individuals who died from paraquat intoxication [31]. For many years, experimental studies using paraquat were focusing on its effects on lung, liver, and kidney, probably because the toxicity induced by this herbicide in these organs is responsible for death after acute exposure [32]. Epidemiological studies in agricultural communities have suggested an increased risk for PD due to paraquat use, raising the possibility that paraquat could be an environmental parkinsonian toxin. This chemical causes extensive oxidative stress in mitochondria of the cell, resulting in the perturbation of biochemical processes, cell death, multi-organ failure, and neurodegenerative diseases [33].

It is still vague about how the molecular mechanism of paraquat leads to cell death. However, studies have shown that paraquat can trigger the sequential phosphorylation of c-Jun N-terminal kinase (JNK), c-Jun, and the activation of caspase-3 consequently leading to neuronal death both in vitro and in vivo [34], suggesting that JNK signaling pathway contributes to paraquat-induced neurodegeneration.

## 2.5. Rotenone

Rotenone is a commonly used natural pesticide prepared from the roots of certain tropical plants, such as *Derris elliptica*. It is a classical high-affinity-specific inhibitor of mitochondrial complex I. This lipophilic compound crosses the blood-brain barrier rapidly and accumulates in subcellular organelles such as mitochondria where it impairs oxidative phosphorylation by inhibiting complex I of the electron transport chain [35]. Postmortem studies implicated mitochondrial impairments [36], and epidemiological studies suggested an association with environmental toxins, in particular mitochondrial complex I inhibitors such as rotenone [37]. *In vitro*, rotenone has been shown to produce cell apoptosis, accumulation, and aggregation of  $\alpha$ -synuclein and ubiquitin, oxidative damage, and endoplasmic reticulum stress [38, 39]. In a study in post mortem idiopathic PD brain, the substantia nigra is seen to comprise of a strong inhibition of complex I activity [40] suggesting this could be the cause of degeneration of dopaminergic neurons.

## 3. Familial/genetic Parkinson's disease

Till date, 15 known genes and 21 loci have been identified for familial PD. Some of the genes are discussed further.

### 3.1. SNCA

In a study involving a large Italian family, Polymeropoulos and colleagues identified the missense mutations in the *SNCA* gene. Through a traditional linkage approach, they managed



to track the underlying genetic injury to an area located in the long arm of human chromosome number 4 [41]. This discovery has been a FRAME shift in the genetic research of PD. A separate study showed that the  $\alpha$ -synuclein protein is the main constituent of the Lewy body which is the pathological hallmark of PD [42]. These two vital research findings brought about a link between sporadic and familial forms of PD.

Five different missense mutations in *SNCA* have been implicated in PD namely A53T, A30P, E46K, H50Q, and G51D mutation. Clinically, early age onset of parkinsonism with a positive initial response to levodopa treatment are seen among patients with missense mutations but later on the disease progresses rapidly with dementia as a common feature. Eventually, appearance of cognitive decline, hallucinations, and fluctuations of consciousness in patients becomes clear. Histopathological study reveals an abundant LB pathology [43].

### 3.2. LRRK2

An association between apparent autosomal-dominant parkinsonism and chromosome number 12 was suggested by findings on a study involving a large Japanese family [44]. Later it was established that mutations in the gene *LRRK2* lead to the basic genetic cause of chromosome number 12 linked PD [45]. The most frequent *LRRK2* mutation is G2019S, detected in approximately 1% of sporadic and about 3–6% of familial PD cases [46]. R1441G is the second most common mutation after G2019S [47]. Most of the *LRRK2* cases described demonstrate LB in the brainstem accompanied by loss of neurons in the SN. Only a minority of cases exhibit neurofibrillary tangle pathology, glial cytoplasmic inclusions, or neuronal nigral loss without LB [45].

### 3.3. PARKIN

An uncommon syndrome characterized by early dystonia and problems from levodopa treatment, osteotendinous hyperreflexia and comparatively slow motor progression was described in Japan in 1973, which is now known to be an autosomal-recessive juvenile parkinsonism (AR-JP) [48]. Mutations in parkin gene was identified as a cause of this condition [49]. AR-JP maps to the long arm of chromosome number 6 and linked to the markers *D6S305* and *D6S253* [50]. It was found that the former is deleted in an AR-JP Japanese patient [51]. By positional cloning within this microdeletion, Kitada and colleagues isolated a cDNA clone of 2960 bp with a 1395 bp open reading frame which code a 465 amino acid protein consisting of an N-terminal ubiquitin-like domain and RING domain having two RING finger motifs. The gene spans more than 500 kb and has 12 exons of which 5 exons (3–7) are found to be deleted in the patient. Also four other AR-JP unrelated patients have a deletion that affect only the exon 4. A 4.5 kb transcript expressed in various human tissues abundantly in the brain, including the substantia nigra, is shorter in brain tissue from one of the groups of exon 4 deleted patients. Therefore inferring that the mutations in this newly identified gene is responsible for the pathogenesis of AR-JP. In a number of families, PD is related with heterozygous *parkin* mutations through an apparently dominant way of transmission, suggesting that the carriers of a sole *parkin* mutation might be at risk to develop the PD [52].

### 3.4. DJ1

A homozygous deletion and a missense mutation in the Daisuke-Junko-1 (*DJ-1*) gene as a cause of autosomal-recessive early onset PD was identified [53]. A number of novel *DJ-1* mutations have been discovered in patients with early onset PD. However, these mutations are rare and can be found in only ~ about 1% of early onset PD cases [54]. At the clinical level, the phenotype of *DJ-1* subjects is the same as that of *parkin* and *PINK1*-related parkinsonism, with age at onset (AAO) usually around the mid-30s, good response to levodopa treatment, slow disease progression, and often focal dystonia such as blepharospasm [55]. However, the neuropathology of *DJ-1*-linked parkinsonism still remains unidentified.

### 3.5. PINK1

Mutations in the phosphate and tensin homolog-induced Putative kinase 1 (*PINK1*) were initially identified in a Sicilian family with autosomal-recessive parkinsonism [56]. In most of *PINK1* mutations, the type of mutation seen is missense. However, mutations of copy number, genetic, and exonic rearrangements have been described [57]. In both the cases of familial and sporadic PD, investigation of mutation has recognized homozygous and compound heterozygous type *PINK1* mutations. This raised the potential role on a single *PINK1* heterozygous mutation to be a possible risk factor for PD [58]. In a number of families, *PINK1* mutations have been associated with early onset PD and *PINK1* mutations association in sporadic cases is about 2–4%. In a clinical phenotype, this type of parkinsonism is quite comparable to those seen among patients with *parkin* and *DJ-1* mutations. They display progressive levodopa-responsive disease gradually [57].

### 3.6. Vacuolar protein sorting-associated protein 35 (VPS35)

Mutation in VPS35 causes monogenic form of PD was described by using exome sequencing in two separate studies with identification of p.D620N mutation in VPS35 among the members of a Swiss kindred with a late onset, autosomal-dominant PD [59]. An independent study published the identification of the p.D620N mutation in a large multi-generation Austrian PD family and in two additional families screened for VPS35 mutations [60]. The VPS35-linked families reportedly fulfill the London Brain Bank criteria for PD, but there are fairly limited clinical and pathological details on these cases.

### 3.7. ATPase (P-type) 13A2 (ATP13A2)

In a neuronal P-type ATPase gene, ATP13A2, a loss of function was initially described in a consanguineous Jordanian family [61]. Clinically, the subjects showed a very early onset of the disease accompanied by rigid-akinetic phenotype with reduced resting tremor, pyramidal syndrome, progressive cognitive impairment, vertical gaze palsy, mini myoclonus, insomnia, and levodopa responsive [61]. Mutations of this gene mapping on chromosome 1p36 cause PD in atypical form which is known as Kufor-Rakeb syndrome [62]. Clinical phenotype of this early onset pallido-pyramidal syndrome varies in severity but only a handful of cases and families have been reported [63, 64].

### 3.8. PLA2G6

Homozygous mutations in phospholipase A2 gene (PLA2G6) located on chromosome 22q13.1 was reported in three patients of two inbred Pakistani families. The phenotypes were associated

with cognitive and psychiatric problems and dystonic features, pyramidal syndrome [65]. In an Asian group with early onset recessive parkinsonism caused by compound heterozygous mutations in PLA2G6, the phenotype reported were frontotemporal lobar atrophy and dementia [66]. The PLA2G6 gene encodes a protein of phospholipase A2 group VI (PLA2G6), which act as catalysis of fatty acids elimination from phospholipids and involved in maintaining membrane phospholipids homeostasis [67].

### 3.9. UCH-L1

Missense mutations of gene coding for the ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), an ubiquitin recycling enzyme located on chromosome 4p14 was reported in a German family with an autosomal-dominant transmission PD [68]. The phenotype of affected individuals was consistent with that of idiopathic PD. An epidemiological study suggested an association between the UCH-L1 gene S18Y variant and PD [69]. Overexpression of UCH-L1 gene upregulated aggresomes formation through dysfunction of proteasome system [70].

### 3.10. HtrA2

Various studies indicate risk factor for parkinsonism due to loss of function of the gene encoding Omi/high temperature requiring A2 mitochondrial protein (HtrA2) in German [71] and Belgians PD patients [72]. The serine protease Omi/HtrA2 is released from mitochondria and promotes apoptosis [73] and mutations of Omi/HtrA2 gene affect its protease activity linked to mitochondrial dysfunction [71, 72]. Although it acts independently of parkin, Omi/HtrA2 functions in the PINK1/parkin pathway downstream of PINK1 [74]. These findings were not confirmed in Omi/HtrA2 knockout mutants in contrast to PINK1 or parkin null mutants [75].

### 3.11. EIF4G1

Mutations in the eukaryotic translation initiation factor 4-gamma (*EIF4G1*) was identified as a risk factor in a study of a northern French family with autosomal-dominant late onset parkinsonism on the chromosome 3q26-q28. Genomic analysis identified a heterozygous mutation in EIF4G1 which was confirmed subsequently in 2 PD patients and 2 Lewy body disease patients among 225 more patients [76]. Further, in all the affected members of another multiplex unrelated family, a pathogenic mutation was detected including in one unaffected 86-year-old family member suggesting an incomplete penetrance [77].

## 4. Animal models of Parkinson's disease

### 4.1. Criteria for modeling PD in animals

Being a neurodegenerative disorder, the prominent hall mark of PD is progressive loss of dopaminergic neurons in the *Substantia nigra pars compacta* (SNpc) [78]. Together with DA neurons, there is a loss of cholinergic neurons, serotonergic neurons, and noradrenergic neurons in the brain [79]. The prominent biomarker being aggregation of Lewy bodies in intracytoplasmic space [80]. A combination of all these features shows the hallmark motor and non-motor symptom of PD.



A suitable model for PD should show histopathologically characterizable progressive loss of DA neurons together with other neurons and significant reduction in DA level. Moreover, the onset of the disease should be in adulthood this should manifest in such a way that it would mimic the PD-affected human motor symptom such as bradykinesia, rigidity, postural instability, and resting tremor, with motor features being responsive to L-DOPA or any anti-PD drug therapy. Even though non human primate and mouse has been the traditional model of PD, because of low cost of maintenance, shorter life cycle, and defined neuropathological profile is making zebrafish and *Drosophila* are among the emerging and more interesting model of PD [81].

## **4.2. Advantages and limitation of modeling PD using drosophila**

### *4.2.1. Advantages*

*Drosophila* offers many advantages for studying human neurodegenerative diseases and their underlying molecular and cellular pathology. Benefits include a faster time frame due to the shorter life span of the fly (10–14 day from pupae to adult), large number of progeny, availability of many techniques, and tools to manipulate gene expression [82]. Also *Drosophila* has been studied for longer than any other model out there which makes its anatomy and phenotypes very well known to experimental biologists [83]. A well developed CNS and prominent number of DA neurons [84] combined with well characterized behaviors which are conserved among strains in 90% cases [85] makes fly very cost effective and efficient model. Genetically, it has been estimated that nearly 75% of disease-related genes in humans have functional orthologs in the fly [86]. While overall similarity at nucleotide or protein level is 40% but in conserved domain it is 80–90% or higher [87].

### *4.2.2. Disadvantages*

The major difference being an invertebrate there will be some prominent difference in physiology with human (e.g., brain anatomy, cardio vascular system, and respiratory system) which relating complex motor behavior with human a difficult task [88]. For CNS-related studies, there is also an issue on blood-brain permeability difference [89]. The metabolic differences are also to be considered when studying drug efficacy and toxin-induced disease phenotype.

## **4.3. Relevance of study of PD in flies**

### *4.3.1. Drosophila mimicking human PD symptoms*

Although the physiological difference between human and flies are very prominent core pathology observed in human PD can be produced in a very accurate extent by toxin-induced or transgenic modification. It is as accurate as area specific and age-dependent DA neuron loss as observed in PD condition and hallmark PD biomarker the LB and LN inclusions are visible in transgenic system [90]. *Drosophila* also performs complex behavior such as mating, aggression, conditioning to fear, learning and motor behaviors such as flying, climbing, and walking [85] which like human are affected by the PD onset and progression. These multitudes of behavior are very much helpful in characterizing different aspects of PD.

#### 4.4. Physiological attributes of fly brain

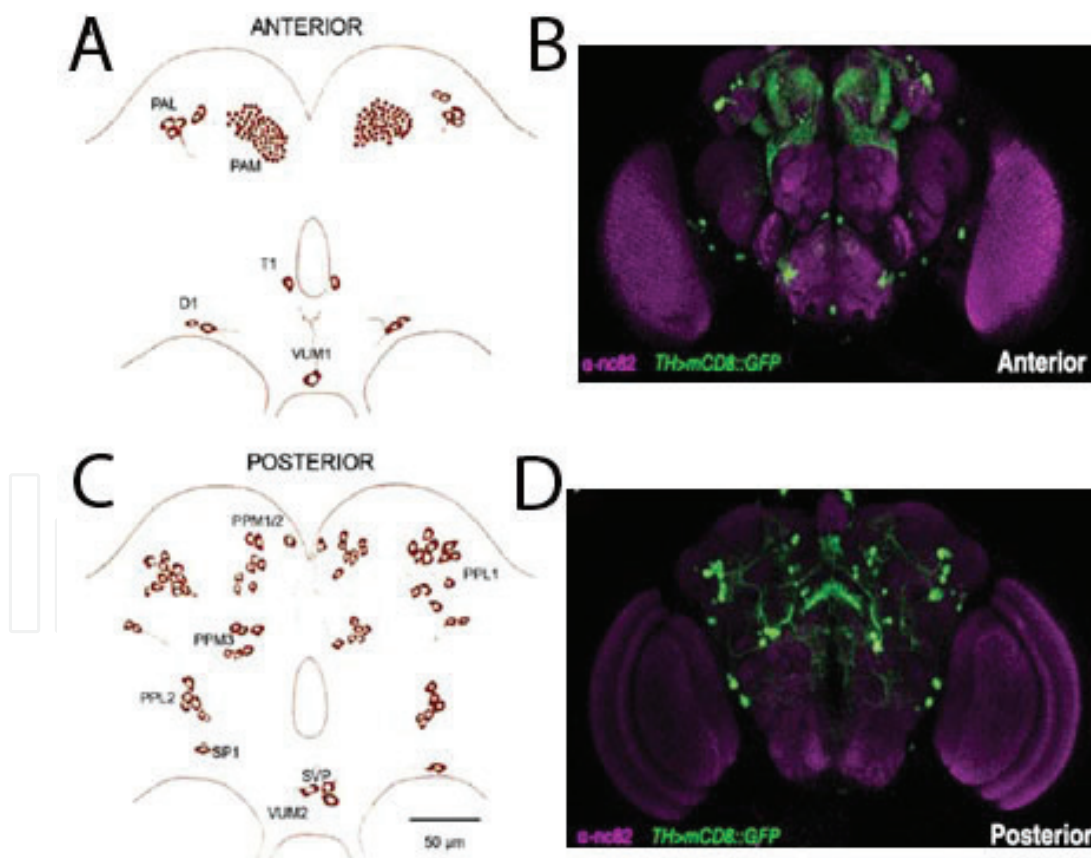
*Drosophila* model of PD has two principle phenotypes: the specific loss of DAergic neurons with aging brain and defects in motor behavior. In fly brain, the DA neurons are distributed as a group of clusters throughout the brain and project their effect on different behavioral patterns of the fly by different functional areas of the adult brain. Target areas include:

- **The mushroom bodies:** involved in memory formation and motivation.
- **The Central Complex:** controls the motor behavior.

The phenomenon of different part of brain controlling different behavior pattern highly resembles the mammalian brain (**Figure 1**) [84].

#### 4.5. Genetic similarity between *Drosophila* and human

*Drosophila* shares 61% homology with human genetically. In fact all the familial or sporadic genes reported so far in human associated with parkinsonism are available in *Drosophila* as a



**Figure 1.** (A and C) Schematic representation of an adult fly brain with the distribution of DA neurons grouped in clusters and arranged with bilateral symmetry (image adapted from Botella et al. [91]). (B and D) Confocal Z-stack of *TH > mCD8::GFP* brain; anti-nc82 immunoreactivity together with GFP labeling reveals dopaminergic neurons in the anterior and posterior brain (image adapted from White et al. [84]).

Gene/protein	Inheritance	Fly homolog	Protein function
$\alpha$ -Synuclein	AD	None	Pre-synaptic protein
Parkin	AR	Parkin/CG10523	E3 ubiquitin ligase
UCH-L1	unclear	Uch/CG4265	E3 ubiquitin hydrolase/ligase
PINK1	AR	Pink1/CG4523	Mitochondrial kinase
DJ-1	AR	DJ-1a/CG6646 DJ-1b/CG1349	Redox sensor/Chaperone
LRRK2	AD	lrrk2/CG5483	Kinase/GTPase
HtrA2	AD	HtrA2/CG8486	Mitochondrial pro-apoptotic protease
GBA	unclear	CG33090	Lysosomal enzyme
POLG	unclear	tamas/CG8987	Mitochondrial DNA polymerase
Tau	unclear	tau/CG31057	Microtubule stabilization

Notes: UCH-L1 = ubiquitin carboxyl-terminal esterase L1; PINK1 = PTEN-induced putative kinase 1; LRRK2 = leucine-rich repeat kinase 2; HtrA2 = high temperature requirement protein A2; GBA = glucocerebrosidase; POLG = polymerase gamma; AD = autosomal dominant; AR = autosomal recessive.

**Table 1.** Showing parkinsonian genes and their fly homologs [92].

homolog. But there is no homolog for the gene  $\alpha$ -synuclein which produces Lewy bodies and Lewy neuritis at the extracellular matrix of brain a hallmark biomarker under PD condition in mammalian brain. Nevertheless  $\alpha$ -synuclein transgenic models respond very well under PD conditions and recapitulate the PD phenotypes. Given (Table 1) are the list of parkinsonian genes and their homologs in flies.

### 5. Gene-environment interaction studies in PD

The identification of PD symptoms subsequent to ingestion of MPTP led to the idea that environmental factors could be related to the causes of pathogenesis of the disease. When ingested, MPTP is metabolized to neurotoxin MPP<sup>+</sup> which was originally developed as an herbicide, cyperquat. The chemical structure of MPP<sup>+</sup> is similar to that of the widely used herbicide paraquat. This finding suggested that exposure to environmental factors such as pesticide, herbicides may contribute to human sporadic PD. Over the years, environmental factors, including pesticides and herbicides, metals, tobacco and caffeine, head injuries, etc. have been largely considered as possible PD risk factors.

Over the last few decades either through genetic or toxin challenges many animal models have been developed to study PD. Using *Drosophila melanogaster* as a model has proved to be of great value and has contributed significantly toward understanding the mechanism underlying PD pathogenesis. Table 2 illustrates the interaction studies between environmental toxins and PD genes using *Drosophila* model.

Gene/protein	Toxin(s)	Observations	Reference
$\alpha$ -Synuclein	Rotenone	<i>Drosophila</i> larvae expressing mutant human $\alpha$ -synuclein showed significant loss of DA neurons and deficit in locomotion	[93]
DJ-1	Paraquat Rotenone	DJ-1 KO flies selectively sensitive to oxidative stress induced by toxins	[94] [95]
LRRK2	Rotenone	hLRRK2 transgenic flies showed loss of DA neurons and sensitivity to environmental toxin	[96]
Parkin	Rotenone	Transgenic flies expressing parkin mutation show age-dependent DA neuron degeneration and locomotor deficits	[97]
PINK1	Rotenone	Flies expressing PINK1 mutations show increased rates of mortality in the mutants	[98]

**Table 2.** Gene-environment interaction studies in drosophila model of PD.

Environmental toxins such as paraquat (herbicide) increases the risk factors for PD and this susceptibility is influenced by the genetic background. Fly model exposed to paraquat shows neurodegenerative symptoms induced by oxidative stress which are similar with most behavioral characters of PD. As seen from epidemiological studies, male flies are more susceptibility to paraquat toxicity than female flies. *Drosophila* mutant for dopamine regulating genes show variable susceptibility to paraquat such as mutations which increase DA pathway function helps in reducing paraquat neurotoxicity while loss of function mutations increases susceptibility to paraquat by decreasing dopamine levels. The loss of function mutation in negative regulator of DA production (Catecholamines-up (Catsup)) acts by delaying the onset of PD symptoms and loss of DA neurons and confers protection against paraquat exposure [99]. *Drosophila* DJ-1 mutants developed motor deficits when exposed to paraquat [100]. Loss of function of DJ-1 $\beta$  mutants confers resistance to paraquat and lowers mortality of DA neuron and overexpression of DJ-1 $\alpha$  in DA neurons, protects against paraquat toxicity [95]. Acute paraquat exposure in *Drosophila* showed elevated levels of oxidative stress markers and mitochondrial dysfunction [101]. Role of ubiquitin proteasome system (UPS) to sporadic PD is not very clear though it is a potential target for PD risk associated with pesticide [102]. Data from epidemiological studies show that paraquat in addition with maneb or ziram increases PD risk [103]. *Drosophila* knockdown of E1 ligase when exposed to paraquat + maneb showed significant DA neuron loss thus imply synergistic effects of the pesticides on risk for PD [104].

Rotenone exposure suppresses proteasomal activity through ATP depletion thus inhibiting mitochondrial function [105]. Parkin through its E3 ligase function offers neuroprotection against neuronal insults including rotenone [106]. Rotenone alters parkin solubility increasing intracellular aggregation and S-nitrosylation of parkin [107]. Loss of parkin increases rotenone-induced DA cell death in mice [108]. *Drosophila* shows negative geotaxis characteristics and rotenone exposure has shown to cause mortality and locomotor defects affecting the climbing ability of flies [109]. Rotenone also inhibits learning and memory functions in fly and the damage caused shows severe effect than those in  $\alpha$ -synuclein A30P mutant [110].

Exposure of adult *Drosophila* to sublethal doses of rotenone causes concentration-dependent locomotor deficiencies, specific dopaminergic neuronal loss, and reduction in the DA levels in flies [111]. Non-motor symptoms such as circadian rhythms in *Drosophila* are also altered following exposure to rotenone [112]. All these studies highlight the utility of drosophila model to understand the gene-environment interaction in PD.

5.1. *Drosophila* PD models and associated tools

*Drosophila* is proved to be one of the important genetic models to study the disease mechanism in PD. Even though with limitations, the fly model enables rapid and elaborate genetic study, providing in depth cell and molecular studies which offers a unique look into the mechanisms and pathways underlying PD pathogenesis [113]. Currently 14 genes for PD have been identified of which 12 genes have homology with *Drosophila*. Loss of function and gain of function analysis using fly model would provide insights into the mechanism of action of these genes associated with PD (Table 3).

5.2. Mitochondrial genetics of PD: insights from drosophila model

*Drosophila* model has made major contribution in the research area of mitochondrial genetics. The early hints of PINK1/parkin on mitochondrial homeostasis came from studies using *Drosophila* model [98, 114, 115]. The most compelling evidence for a mitochondrial etiology of

Gene/symbol	Drosophila homolog	(Over)Expression construct	(Over)Expression construct with point mutation	Loss of function mutants
SNCA/ PARK1	No	YES	YES	NO
PARK2 encoding parkin/ PARK2	Parkin	YES	YES	YES
UCHL1/PARK5	Uch	NO	NO	NO
PINK1/PARK6	Pink1	YES	YES	YES
PARK7 encoding DJ1/PARK7	Dj-1 $\alpha$ and Dj-1 $\beta$	YES	NO	YES
LRRK2/PARK8	Lrrk	YES	YES	YES
ATP13A2/PARK9	CG32000	YES	NO	NO
HTRA2/PARK13	Htra2	YES	YES	YES
PLA2G6/PARK14	iPLA2-VIA	NO	NO	YES
FBOX7/PARK15	No homolog	YES	YES	NO
VPS35/PARK17	Vps35	YES	Yes	YES
EIG4G1/PARK18	eIF4G	No	NO	NO
DNAJC6/PARK19	auxillin	YES	NO	YES
SYNJ1/PARK20	Synj	YES	YES	NO

Table 3. *Drosophila* model of Parkinson’s disease (genetic) (adapted from Vanhauwaert and Verstreken [113]).



PD was derived from the study of genes mediating familial forms of the disease in fly model [116, 117]. Mutations in *Pink1* (*PARK6*), which encodes a serine-threonine kinase localized to mitochondria and *parkin* (*PARK2*), which encodes a RING finger-containing E3 ubiquitin ligase have been found in recessively inherited and sporadic PD cases [56, 118]. *Drosophila* PINK1 and parkin function in the same genetic pathway, with *pink1* acting upstream of parkin, to regulate mitochondrial integrity in testes, muscle, and dopaminergic neurons [98, 115, 119]. Flies lacking PINK1 or parkin function are viable and show muscle degeneration and TUNEL staining, indicative of cell death [98, 115, 119]. Parkin and PINK1 mutant flies show locomotor defects, abnormal wing position, and dented thoraces [98, 115, 119]. Mitochondrial defects seen in parkin or PINK1 mutant flies are majorly found in the muscle cells though other cell types like DA neurons in the fly brain also show mitochondrial defects which suggests that loss of PINK1 or parkin would result in systemic mitochondrial defects but not extended to all tissues with similar extent [98, 115, 119]. PINK1 and parkin mutant flies show very similar phenotypes suggesting that these genes act together in protecting mitochondria from damage. *Drosophila* expressing wild-type parkin in a PINK1 mutant partially rescues PINK1-associated phenotypes. Alternatively, PINK1 wild type expression in a parkin mutant does not rescue the parkin-associated phenotypes which shows that parkin acts downstream of PINK1 [98, 115]. Parkin is also associated with degradation of mitochondria by autophagy and proteasomal degradation of mitochondrial components [113, 120].

Mitochondrial DNA mutations were first associated with different sporadic or maternally inherited neuromuscular disorders [121]. These disorders were characterized by either the accumulation of multiple mtDNA deletions in post-mitotic tissues [122] or tissue-specific mtDNA depletion [123], and a genetic defect affecting nuclear genes involved in mtDNA replication and maintenance [124]. Mitochondrial DNA often exists in a state of heteroplasmy (cells carrying mtDNA of different genotypes), in which mutant mtDNA co-exists in cells with wild-type mtDNA. Pathology is seen when the frequency of such a mutation reaches a threshold [125]. It accumulates throughout life and is thought to contribute to diseases of aging that include neurodegeneration, metabolic disorders, cancer, heart disease, and sarcopenia [126, 127]. A new investigation of mtDNA in the dopaminergic neurons [128] expanded the previous results showing a prevalent deletion in single neurons on a background of multiple mtDNA deletions [129]. It showed that complex I and complex II are most consistently affected in single neurons, which also displayed a reduced mtDNA copy number [128]. Stimulation of autophagy, activation of the PINK1/parkin pathway, or decreased levels of mitofusin results in a selective decrease in lethal mtDNA deletion [130]. Similar to flies lacking parkin, the flies with mtDNA deletions display striking mitochondrial abnormalities such as disrupted cristae resulting in reduced ATP levels leading to apoptosis and subsequently neurodegeneration.

These dynamic processes regulate mitochondrial function by enabling mitochondrial recruitment to critical subcellular compartments, content exchange between mitochondria, mitochondrial shape control, mitochondrial communication with the cytosol, and mitochondrial quality control making the mitochondria readily adapt to changes in cellular requirements. When mitochondrial dynamics is disrupted, cellular dysfunction follows. The view of mitochondrial dynamics has expanded from a curious phenomenon into an integral cell biological process influencing many cellular functions and survival [131].

### 5.3. Mitochondrial dynamics: fusion and fission

Mitochondria undergo frequent fission and fusion events contributing to the filamentous and interconnected morphology of the organelles and serve crucial physiological functions [132]. The first mitofusin was discovered in mutant *Drosophila* characterized by male sterility [133]. During cell division, mitochondrial fission plays an important role for proper inheritance of mitochondria to the daughter cells. Moreover, organelle fission appears to be important to meet the energy demands of cells at particular subcellular locations. This is true especially for neurons, which heavily depend on energy supply, where mitochondria can travel via microtubule-associated motor proteins to serve specialized neuronal functions such as synaptic transmission. Furthermore, an interconnected network of contiguous mitochondrial organelles seems to be required for the intramitochondrial exchange of metabolic substrates as well as the maintenance of respiratory capacity and mitochondrial membrane potential in hypoxic cellular regions [134].

Mitochondrial fission and fusion events are regulated and coordinated by evolutionarily highly conserved molecular machineries. The molecules regulating mitochondrial dynamics include the homologous GTPases Mitofusin 1 (Mfn1) and Mitofusin 2 (Mfn2), which mediate fusion of the mitochondrial outer membrane, and Optic atrophy 1 (Opa1), a GTPase required for fusion of the inner membrane. Mitochondrial fission, conversely, requires Dynamin-related protein 1 (Drp1), which is also a GTPase [131, 135]. The *Drosophila melanogaster* genome encodes two homologs of Mfn, one being Fuzzy onion (Fzo) [133]. The expression of Fzo is restricted to the testes, and mutations in *fzo* causes mitochondrial fusion defects in testes and male sterility [133]. The second *Drosophila* Mfn homolog is a largely uncharacterized protein known as mitochondrial assembly regulatory factor (Marf), which is expressed in germline and somatic cells [136]. The *Drosophila* genome also encodes single homologs of *opa1* [137] and *drp1* [138], both of which have been shown to function in mitochondrial dynamics in flies.

### 5.4. Genome-wide association studies (GWAS) and genetic screens

GWAS are large-scale population-based genotyping studies that were designed to capture common genetic risk loci and searches for small variations, called single nucleotide polymorphisms or SNPs, which occur more frequently in people with particular disease than in people without the disease. These approaches lead to the identification of new disease causing genes, new biological pathways to explain disease origin or progression, and potential therapeutic targets which is much more precise than the corresponding information from linkage-based studies.

Maraganore and colleagues conducted the first GWAS for PD. It had limited sample sizes (few hundred patients) and patient-control series were sometimes mismatched, but they remain in the genetic history of PD and suggested the low heritability of PD generating a large amount of genetic data into public domain to be further examined and completed by other researchers [139]. Over the last two decades, human GWAS have begun to reveal the genetic risk factors for countless common disorders with complex genetic etiologies including most of the major causes of morbidity and mortality in the developed world [140].

Involvement of multiple genes and pathways complicate the identifying and developing effective therapeutic strategies in different geographical and ethnically divergent populations. Here lies the necessity and importance of GWAS relating to complex neurological disorders such as PD and cancers. Mutations in five genes have been identified to contribute to Mendelian forms of PD risk in fewer than 5% of those with PD which suggest that additional genes too contribute to disease risk [141]. In 2009, two GWAS papers provided unequivocal evidence for an association of the MAPT locus and SNCA variations with sporadic PD. Additionally, both papers implicated variants close to LRRK2 and at two new loci on chromosome 1 (1q32) and chromosome 4, close to the bone marrow stromal cell antigen 1 (BST1) gene [142]. Shortly after these findings, Pankratz et al. conducted the first GWAS in familial PD, confirming the previous discoveries and providing preliminary evidence for an association of a new locus containing the genes cyclin G association kinase (GAK) and diacylglycerol kinase theta (DGKQ) with PD [142, 143]. A meta-analysis on more than seven million polymorphisms originating either from GWAS datasets and/or from smaller scale PD association studies was performed, where 10 loci showed genome-wide significant association with PD risk (*BST1*, *CCDC2/HIP1R*, *DGKQ/GAK*, *GBA*, *LRRK2*, *MAPT*, *MCCC1/LAMP3*, *SNCA*, *STK39*, and *SYT11/RAB25*) and novel evidence for genome-wide significant association with polymorphism in *STGA8* was found [144]. To date, the largest GWAS was performed in 2014 carrying out a meta-analysis in all existing European-ancestry PD GWAS data with 13,708 PD cases, 95,282 controls and a replication study using genotyping array called 'Neuro X' in an independent data set identified 26 independent SNPs which showed genome-wide risk for PD [145]. The first papers about the potential impact of risk loci on age at onset (AAO) in PD were published in 2015. The results using polygenic score analysis showed that patients with an early AAO had a significantly higher polygenic score when compared to those with late AAO [146]. GWAS showed a genetic association with PD in the HLA region (chromosome 6p21.3), which was designated PARK18 and the common variant associated with late onset sporadic PD was rs3129882 in intron 1 of HLA-DRA [147]. GWAS sporadic PD was performed and sterol regulatory element binding transcription factor 1 (SREBF1) was identified as risk factor for PD [148]. Later, an unbiased approach on genome-wide RNAi screen was performed in *Drosophila* cells and validated in HeLa cells to identify genes regulating the PINK1/parkin pathway, which act in a common genetic pathway in mediating the autophagic degradation of mitochondria (mitophagy) and found 20 genes that have a conserved function in promoting translocation and degradation of depolarized mitochondria. But most notable genes involved were (SREBF1) Fbox and WD40 domain protein 7 (FBXW7), and other components of the sterol regulatory element binding protein (SREBP) lipogenesis pathway indicating a role of lipids in mitochondrial homeostasis, which further showed that this pathway regulate mitophagy and also share a common mechanistic link between autosomal-recessive and sporadic PD [149].

The loci currently associated with PD account for only a very small amount (3–5%) of the expected heritability of PD, suggesting that additional heritable factors (genetic or epigenetic) also play a role in transforming susceptibility to PD. While current evidence suggests that common genetic variants play a role in the etiology of typical PD. GWAS by their inherent design may not be able to detect rare variants [143, 150]. Most SNPs detected by GWAS are not likely causal variants for disease risk but rather informative markers hence it is often not

productive to study their direct functional consequences. Also cases selected for GWAS may not be particularly enhanced with genetic susceptibility alleles, moreover the effect sizes identified for most variants are reduced. Thus multiple approaches including linkage analysis, sequencing, and sibpair analysis would be needed to discover additional variants/causative genes and susceptibility loci. Large-scale genome and exome sequencing in conjunction with denser genotyping in large cohorts may help to identify the loci that contribute to the “missing heritability” previously unnoticed by earlier generation technologies [151].

## 6. Utilizing *Drosophila* to understand human GWAS signals

### 6.1. GAL4-UAS system

In 1993, Brand and Perrimon developed the GAL4-UAS system for precise spatial and temporal patterns directing gene expression in *Drosophila* and has been considered as a powerful research tool. A bipartite approach in which a transcriptional activator, the GAL4 gene binds to specific cis-enhancer elements, upstream activation sequence (UAS) leading to activation of the adjacent gene, and thousands of GAL4 driver lines available from individual labs and public stock collections allow expression of desired target genes, typically cDNA transgenes under control of upstream activating sequence (UAS) sites [152]. Findings showed that several familial forms of parkinsonism result from increased gene dosage of  $\alpha$ -syn, based on this expression levels of the  $\alpha$ -syn transgene was augmented by generating an  $\alpha$ -syn expression construct bearing sequence alterations designed to improve the translational efficiency of this cDNA in *Drosophila* and further maximized  $\alpha$ -syn protein expression in the fly brain by making use of flies bearing two copies each of the UAS- $\alpha$ -syn transgene and the TH-GAL4 driver; these control approximately doubled the abundance of  $\alpha$ -syn protein relative to flies bearing a single copy of each of these transgenes generating a more robust *Drosophila* model for studying synucleinopathies [153].

### 6.2. RNA interference (RNAi)

RNAi is an RNA-dependent gene-silencing process that is regulated by the RNA-induced silencing complex (RISC) and triggered by short double-stranded RNA (dsRNA) molecules.

Efficient silencing of gene expression by dsRNA was first discovered by Fire and Mello [154]. RNAi silencing of a specific target gene relies on the ability of small interfering RNAs (siRNAs), long double-stranded RNAs (dsRNAs), or short hairpin RNAs (shRNAs) to target mRNA molecules for degradation [155–157]. *Drosophila* in vivo RNAi techniques screen both the whole genome and subsets of genes. A total of 10,689 different genes (78% of the *Drosophila* genome) were assayed that affect susceptibility to intestinal *Serratia marcescens* infection [158]. Of these, 8.3% (885 genes) were defined as hits; the majority 89.3% (790 genes) were susceptibility candidates, and 95 genes (10.7% of hits) were negative regulators. A total of 78 and 56 genes were found to function only in the gut and hemocytes, respectively, and 79 functioned in both. A primary screened of 6923 UAS-IR strains for genes involved in the glycosylation of a neural glycoprotein and identified 171 candidates [159]. These were further confirmed by



knock down experiments, using in-silico analysis and a secondary set of UAS-IR strains that targeted regions distinct from those of the primary strains. A total of 2970 genes were knocked down by neuron-specific RNAi in search for genes involved in the formation, growth, and maintenance of the neuromuscular junction (NMJ) [160]. Knockdown of 158 genes in post-mitotic neurons led to abnormalities in the neuromuscular system. Genome-wide small interfering RNA (siRNA) screening yielded gene candidates involved in characterization of TOMM7 required for stabilizing PINK1 on the outer mitochondrial membrane following mitochondrial damage. Also, HSPA1L (HSP70 family member) and BAG4 found to have mutually opposing roles in the regulation of parkin translocation. RNAi screens revealed that SIAH3 localize to mitochondria, inhibits PINK1 accumulation after mitochondrial insult, and reducing parkin translocation. Overall, screens provide a rich resource to understand mitochondrial quality control [161].

Using this inducible RNAi technique, large-scale screens for various biological processes have been performed successfully in *Drosophila*, proving RNAi-based in vivo screen adequate and efficient. However, RNAi-based screens have relatively high levels of false positives and negatives. To validate the screening results, experimental, and computational analyses have been proposed which will increase the accuracy of RNAi-based screen results.

### 6.3. CRISPR/Cas9

Clustered regularly interspaced short palindromic repeat/CRISPR-associated protein 9 (CRISPR/Cas9) was first applied in mammalian cells in 2013 which has been used as an essential tool in biotechnology [162]. The CRISPR/Cas9 system is a novel genome modification method in which gRNA direct the nuclease Cas9 to selected sequences of genomic DNA, and Cas9 cuts both strands at a specific location. Non-homologous end joining (NHEJ) or homology-directed repair (HDR) repairs the genomic DNA resulting in mutations that can interrupt the open reading frame and cause gene inactivation. For example, loss in function of parkin and Pink1 genes causes PD, CRISPR/Cas9-mediated mutations can mimic knockout of the parkin and/or Pink1 gene [163]. Thus, when both alleles are mutated by CRISPR/Cas9, the complete loss of parkin or Pink1 will mimic the genetic mutations in PD patients. Indeed, CRISPR/Cas9 has been used to generate pig models of PD by targeting the genes for parkin, Pink1, and DJ1 [164]. In addition to genome editing in germline cells, CRISPR/Cas9 can efficiently target genes in somatic tissues, such as neurons in the brain [165, 166]. In PD patients, continuous loss of dopaminergic neurons in the substantia nigra is a fundamental pathological feature. Thus, gRNAs and Cas9 can be delivered to the substantia nigra of animal brains by a viral system to examine the effect of parkin or Pink1 loss in adult brains. This approach is especially useful for investigating the age-related neuropathology in PD. Also, by gaining toxicity of mutant proteins Cas9-mediated knock-in mutations within the genome can develop animal models of those neurodegenerative diseases.

For transgenic PD animal models that express mutant  $\alpha$ -syn, CRISPR/Cas9 can be designed to reduce the expression of mutant genes via NHEJ, which can lead to gene inactivation, in dopaminergic neurons. Besides, replacing the mutant gene by CRISPR/Cas9 via HDR with normal DNA sequences can also lead to the genetic correction of DNA mutations in PD animal



models. Even though efficiency of such gene replacement is low at present, the rapid development of CRISPR/Cas9 system offers a promising attempt to produce knock-in models of human diseases [167].

#### 6.4. Deciphering the pathways of therapeutic molecules: role of drosophila model

The first published study about compound treatments in a drosophila PD model reported the effects of drugs commonly used for treating PD on the locomotor phenotype of  $\alpha$ -synuclein expressing flies and showed that some of them were able to suppress that phenotype [168]. Subsequently, and given the ability of increased chaperone activity to counteract  $\alpha$ -synuclein toxicity [169], the effect of geldanamycin (GA), an antibiotic able to interfere with Hsp90 activity and activate stress response, was assayed over  $\alpha$ -synuclein expressing flies [170, 171]. Notably, feeding these flies with GA protected DA neurons against  $\alpha$ -synuclein-induced degeneration, and this protection was driven by an increase in Hsp70 levels [171]. Inhibitors of the histone deacetylase SIRT2 also showed a protective effect against  $\alpha$ -synuclein toxicity [172]. Other studies have been also performed in several drosophila PD models to look for potentially therapeutic compounds directed to reduce oxidative stress damage. As explained previously, the study of  $\alpha$ -synuclein toxicity in flies led to the identification of Phase II detoxification pathway as a possible target for therapeutic treatment [173]. In fact, feeding  $\alpha$ -synuclein-expressing flies or *Drosophila parkin* mutants with pharmacological inducers of that pathway like sulforaphane or allyl disulfide suppresses the neuronal loss of both PD models [173]. Besides, it has been shown that dietary supplementation with S-methyl-L-cysteine (SMLC) inhibits the locomotor and circadian rhythm defects caused by ectopic expression of human  $\alpha$ -synuclein in drosophila [174]. SMLC participates in the catalytic antioxidant mechanism involving methionine sulfoxide reductase A (MSRA), one of the enzymes that catalyze the oxidation of the amino acid methionine to methionine sulfoxide, a reversible reaction that has been postulated to act protecting cells from oxidative damage. Furthermore, grape extract supplementation has been shown to recover locomotor ability and lifespan in  $\alpha$ -synuclein-expressing flies. It is known that grape extracts contain several polyphenols, compounds with antioxidant properties [175]. Other drosophila PD models in which treatments with antioxidant compounds have been shown to be beneficial are those involving the *DJ-1 $\alpha$*  and *DJ-1 $\beta$*  genes [176, 177]. Compounds with antioxidant and anti-inflammatory properties such as celastrol and minocycline conferred potent DA neuroprotection in RNAi *DJ-1 $\alpha$*  mutants [176]. We have also recently demonstrated that chronic treatments with antioxidant compounds are able to modify the lifespan phenotype of *DJ-1 $\beta$*  mutant flies, thus suggesting that oxidative stress plays a causal role in such phenotype [177]. It is known that rapamycin is a small molecule inhibitor of TOR signaling that has been shown to lead to 4E-BP hypophosphorylation in vitro and in vivo [178, 179]. Notably administration of rapamycin was able to suppress all pathologic phenotypes in *parkin* and *PINK1* mutants. Moreover, this suppression was found to be 4E-BP dependent, since the administration of rapamycin to *parkin* and *Thor* or *PINK1* and *Thor* double mutants was completely unable to suppress these phenotypes [180]. Since 4E-BP activity can be manipulated by small molecule inhibitors such as rapamycin, this pathway represents a viable therapeutic target for PD treatment. Moreover, it has been recently suggested that *parkin* mutants, apart from the described phenotypes, also present altered zinc homeostasis. This is supported by

the fact that dietary zinc supplementation in the form of zinc chloride increased lifespan as well as the percentage of *parkin* mutant flies reaching adulthood while this supplemented diet was deleterious to control flies [181]. Since most PD cases are sporadic and could be associated to different environmental agents, it is also essential the use of toxin-induced *Drosophila* PD models to assay the beneficial effects of candidate compounds. Polyphenol administration was also found to exert a beneficial effect on flies exposed to paraquat and iron, protecting, rescuing, and restoring the impaired locomotor activity caused by exposure to those agents [182]. Other antioxidant compounds such as melatonin have also been found to rescue locomotor deficits and DA neurodegeneration in flies exposed to rotenone [19].

#### 6.4.1. LRRK2 kinase inhibitors

Several LRRK2 kinase inhibitors, including CZC-25146, GW5074, and sorafenib, have been tested in rodents, as well as in *Caenorhabditis elegans* and *Drosophila* models, and have been shown to protect against LRRK 2 (G2019S)-induced neurodegeneration [183]. These findings indicate that increased kinase activity of LRRK2 is neurotoxic and that inhibition of LRRK2 activity can have a disease-modifying effect.

#### 6.4.2. Molecular chaperones

##### 6.4.2.1. HSF-1 modulators

Endogenous molecular chaperone function can be modulated pharmacologically with compounds that augment endogenous chaperone levels. Several HSF-1 modulators including celastrol and carbenoxolone can trigger HSF-1 activation, leading to downstream induction of Hsp70 expression [184]. Celastrol has been demonstrated to be effective against protein aggregation and toxicity in various neurodegenerative disease models, including dopaminergic neuroprotection in a *Drosophila* model of PD [176]. Carbenoxolone has demonstrated the ability to attenuate  $\alpha$ -synuclein and ubiquitin aggregation in vitro and in vivo [185, 186]. Thus, it may have potential as a chaperone-mediated therapeutic option for PD. Hsp90 Inhibitors a naturally occurring small molecule antibiotic, geldanamycin (GA), inhibits the interaction between Hsp90 and HSF-1, leading to increased Hsp70 expression [187]. In vitro cell studies have demonstrated the capability of this compound to decrease  $\alpha$ -synuclein aggregation and reduce cell toxicity [188], and its neuroprotective effects have been shown in *Drosophila* and MPTP mouse models of PD [169, 189]. Other analogues of GA include 17-AAG and 17-DMAG, which similarly prevent  $\alpha$ -synuclein aggregation and toxicity, but are more potent and less toxic than GA [190]. Moreover, 17-AAG has poor permeability of the BBB, limiting its pharmacological usage for neurodegenerative diseases [185]. Consequently, compound library screening for small molecule Hsp90 inhibitors with improved pharmacokinetics, including BBB permeability, have led to the identification of SNX compounds [185]. These compounds are associated with an increase in Hsp70 activity in the brain and a reduction in  $\alpha$ -synuclein oligomerization and toxicity in vitro [190].

Insights regarding identification of pathways through which different therapeutic molecules confer neuroprotection is briefed in **Table 4**.

Pathway/process	Compound treatment*	Drosophila model	Modified phenotype/s	References
Oxidative stress	Sulforaphane and allyl disulfide*	<i>Parkin</i>	DA neuron number	[173]
		$\alpha$ -synuclein	DA neuron number	[173]
	S-methyl-L-cysteine	$\alpha$ -synuclein	Locomotor activity	[174]
	Polyphenols	$\alpha$ -synuclein	Lifespan, locomotor	[175]
	$\alpha$ -tocopherol	Paraquat and iron	activity	[182]
	SOD	DJ-1 $\beta$	Locomotor activity	[177]
	Melatonin	PINK1	Lifespan	[191]
	<i>Bacopa monieri</i> leaf extract	PINK1	Ommatidial	[191]
		DJ-1 $\beta$	degeneration	[177]
		Paraquat	Ommatidial	[99]
		Rotenone	degeneration	[99]
		Paraquat	Lifespan	[192]
			Locomotor activity	
			Locomotor activity, DA neuron	
			Oxidative markers levels	
Oxidative stress/ inflammatory process	Minocycline*	DJ-1 $\alpha$	DA neuron number,	[176]
	Celastrol		dopamine Levels	
			DA neuron number,	
			dopamine levels,	
			locomotor activity, and	
			survival rate under	
			oxidativestress condition	
TOR signaling	Rapamycin*	<i>parkin/PINK1</i>	Thoracic indentations, locomotors activity, DA neuron number, and muscle integrity	[180]
Removal of excess or toxic protein forms	Geldanamycin*	$\alpha$ -synuclein	DA neuron number	[170]
				[171]
Zinc homeostasis	Zinc chloride*	<i>parkin</i>	Lifespan, locomotor activity, and percentage of adulthood survivors	[181]
Chaperone therapies (HSF-1 modulators) Trigger HSF-1 activation Induces downstream Hsp70 expression	Celastrol	$\alpha$ -synuclein	dopaminergic	[184]
	Carbenoxolone	$\alpha$ -synuclein	neuroprotection	
Hsp90 inhibitors Inhibits the interaction between Hsp90 and HSF-1, leading to increased Hsp70 expression and activity	Geldanamycin	$\alpha$ -synuclein	decrease $\alpha$ -synuclein	[187]
	17-AAG		aggregation and reduce	[190]
	17-DMAG		cell toxicity	
	SNX-2112			
mTOR-dependent pathways/AMPK	Metformin AICAR	<i>Drosophila melanogaster</i> mutated for LRRK2	Reduced cell death	[193]
mTORC1	Rapamycin and Rp analogues (CCI-779, RAD001 and AP23573)	<i>Drosophila melanogaster</i> mutated for PINK-1 and Parkin	Reduced mitochondrial Dysfunction	[180]

Pathway/process	Compound treatment*	Drosophila model	Modified phenotype/s	References
mTor-independent pathways/unknown	Spermidine	<i>α-synuclein</i>	Reduced motor dysfunction, increased lifespan; Reduced neuronal cell loss	[194]
LRKK2 kinase inhibitors	GW5074, and sorafenib	<i>α-synuclein</i>	Protect against DA neuron degeneration locomotor activity	[183]
Histone Deacetylase inhibitors		<i>α-synuclein</i>	Protect against DA locomotor activity	[195]
Antitumor agents	Geldanamycin	<i>α-synuclein</i>	Protect against DA Mobilized the stress response and increase levels of chaperon HSP70	[195]

\*All treatments were administered as dietary complement.

**Table 4.** Listing of pathways through which neuroprotective compounds confer neuroprotection: lessons from drosophila model of PD.

## 7. Conclusion

**Bench to bedside:** role of drosophila in translational research.

Bench to bedside is a term used to describe the process by which the results of research done in the laboratory are directly used to develop new ways to treat patients. Taking advantage of studies from animal models such as drosophila certain pharmacotherapies and non-pharmacotherapies have been developed which are in different stages in clinical trials to validate their efficacy, safety, and tolerability. Pharmacotherapies include adenosine A2A receptor antagonists [196], glutamate AMPA receptor antagonists [197], monoamine oxidase inhibitors [198], anti-apoptotic agents [199], and antioxidants [200]. Non-pharmacotherapies also offer alternative approaches for treatment of the disease which include the use of viral vector gene therapy [201], stem cell transplants [202], and microRNAs [203]. Nevertheless, additional trials enrolling larger numbers of PD patients are still needed to better understand the neuroprotective effects of these therapies.

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## Conflict of interest

Authors declares no conflict of interest.

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