Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 3343; doi:10.3390/ijms19113343

Drosophila models of sporadic Parkinson's disease

Emi Nagoshi

Department of Genetics and Evolution, Sciences III, University of Geneva, 30 Quai Ernest-Ansermet, Geneva-4, CH-1211, Switzerland

Correspondence: Emi.Nagoshi@unige.ch

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

Abstract

Parkinson's disease (PD) is the most common cause of movement disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra. It is increasingly recognized as a complex group of disorders presenting widely heterogenous symptoms and pathology. Except for the rare monogenic forms, the majority of the PD cases result from an interaction between multiple genetic and environmental risk factors. The search for these risk factors and the development of preclinical animal models are in progress hand in hand, providing mechanistic insights into the pathogenesis of PD. This review summarizes the studies that capitalize on modeling sporadic (i.e. non-familial) PD using *Drosophila melanogaster* and discuss their methodology, new findings and future perspective.

1. Introduction

Parkinson's disease (PD) is the second most frequent neurodegenerative disorder, affecting seven to 10 million people worldwide. It presents with motor symptoms, such as bradykinesia, rigidity, postural instability and resting tremor, but also manifests a broad spectrum of non-motor symptoms, including sleep disturbances, mood disorders and cognitive impairments [1, 2]. A prominent pathology of PD is loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc), which project to the striatum. The resulting striatum DA deficiency leads to the characteristic motor dysfunction. Another histopathological hallmark of PD is the presence of abnormal aggregates (inclusions) containing α-synuclein (α-syn) protein, termed Lewy bodies and Lewy neurites [3], although many PD cases without Lewy body pathology have been reported [4, 5]. No treatment strategy to cure or even slow

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

down PD progression is currently available. With the prevalence of approximately 1% at 60 years old [6], better understanding of PD pathogenesis for developing an improved treatment strategy is a growing need in aging societies.

PD was long considered as a non-hereditary disease; only approximately 15% of PD patients have a family history and no more than 10% of cases are Mendelian forms of PD [1, 7]. The rest of the cases are defined as "sporadic PD", the disease case occurring in people with no positive family history of PD. This does not, however, exclude genetic factors as causative agents. It is most likely of multifactorial origin, resulting from complex interactions of genetic and environmental factors. Sporadic PD can be monogenic, because a dominant mutation may have occurred *de novo* or the mutation is recessive. Except for the monogenic forms of familial PD, which are frequently early-onset [8, 9], familial and sporadic PDs are clinically indistinguishable [10, 11]. Then, how do we go about modeling sporadic PD? Are the models for familial and sporadic PDs fundamentally different or based on similar strategies? To what extent do familial PD models provide knowledge relevant for sporadic cases and *vice versa*? This review aims to address these questions by introducing the literature, in which sporadic PD models were established or used for mechanistic or descriptive studies in *Drosophila*.

2. How to model a disease that occurs sporadically?

Sporadic PD, also called as idiopathic PD, is by definition the PD case whose cause is yet unknown. As such, it is intrinsically difficult to generate models through an approach targeting the "causal" genes or agents. Nonetheless, the recent progress in identifying candidate genetic and environmental risk factors for PD has made it possible to engineer animal models that carry genetic variations thought to increase

Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 3343; doi:10.3390/ijms19113343

risk of PD, in the same way as most genetic models of familial PD were established. The analogous approach is the exposure to chemical agents known to be associated with increased risk of PD. In addition, there can be a third category of sporadic PD models, which are developed by serendipity, such as the discovery of a mutant animal that exhibit phenotypic similarity to PD. Furthermore, as sporadic PD is likely to be multi-factorial, any combination among these strategies may result in a valid model.

On evaluating the value of animal models, it is worthwhile to perform a mental exercise imagining two extreme theoretical scenarios:

(A) A genetically engineered animal model perfectly recapitulates a known risk genetic variant for PD but shows no characteristics similar to those of PD.

(B) A mutant animal was discovered to exhibit phenotypes similar to cardinal characteristics of PD but the mutant allele/gene is not known to be associated with PD risk.

Which one can be considered as a model for sporadic PD?

In theory, an ideal model should possess the similarity with the human disease in (1) genetic basis, (2) pathological responses, (3) phenotypic endpoints, (4) underlying mechanism(s), and (5) response to known drugs with clinical efficacy. It should be also (6) predictive of human response to drugs [12]. However, it is now a consensus in many disease research fields that no single animal model is expected to possess all these features. The above two scenarios exemplify this limitation. The lack of phenotypic similarity in (A) and known genetic association with PD in (B) may be seen as weakness; however, given the heterogeneity and the multi-factorial nature of sporadic PD, one can interpret the lack of phenotype in (A) as missing interacting genetic or environmental factors for developing PD and speculate the existence of rare genetic variants similar to that in (B) in human population. In other words, the

Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 3343; doi:10.3390/ijms19113343

seeming weakness can offer new opportunities for discovery leading to a better understanding of the pathophysiology and underlying mechanisms of the disease. Therefore, I take a liberal position and propose to consider the animal models that possess any of the above criteria (1) to (6) legitimate. On the basis of this premise, here this review recognizes animal models possessing one of the following features as sporadic PD models:

- Based on chemical or genetic agents known to be associated with PD risk
- Show functional impairments or loss of DA neurons, age-dependently
- Exhibit behavioral abnormality similar to the movement disabilities in PD
- Responsive to symptomatic anti-PD medication such as L-dopa
- Show Lewy body pathology

These models can be roughly classified into three categories by the methodology: (1) chemically induced models, (2) targeted genetic models, and (3) serendipitous genetic models. The approach combining multiple methods within and among these categories to create more realistic models is also emerging with success.

3. Why use *Drosophila* for Parkinson's disease research?

This review focuses on *in vivo* models of sporadic PD in *Drosophila melanogaster*, a model organism offering numerous advantages for biomedical research. Its genome is smaller with much less gene redundancy than that of mammals, while at the same time contains homologs of approximately 70% of human disease-related genes [13]. Advanced genetic tools enable mutagenesis, silencing and overexpression of gene-of-interest in cell-type specific and temporally controlled manner [14]. Flies are amenable for large-scale genetic and chemical screening, offering opportunities for

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

not only understanding genetic and molecular basis of diseases but also for drug discovery [15].

The fly's nervous system is simpler but shares many features in structure, organization and function with mammalian counterpart. Flies exhibit various DA-dependent behavior controlled by different subclasses of DA neurons (Fig. 1), whose individual roles and connectivity are unfolding [16, 17]. Startle-induced climbing assay and its variations (such as startle-induced negative geotaxis; SING) are the most commonly used methods to quantify fly's locomotor behavior in comparing organismal phenotypes of flies and human movement disorders [18-21]. These assays are distinct from recording the natural tendency to climb, known as negative geotaxis, but monitor the rate of climbing response of flies when knocked down to the bottom of a cylinder. The climbing response is normally rapid, and failure to recover from the "shock" of being knocked-down suggest some defects in the functioning of nervous system in controlling initiation. Noteworthy, recent studies have demonstrated that at least a subset of DA neurons in the protocerebral anterior medial (PAM) cluster has important roles in the climbing response, much like DA neurons in mammalian SNpc, making flies ever relevant model system for the study of PD [18, 22, 23].

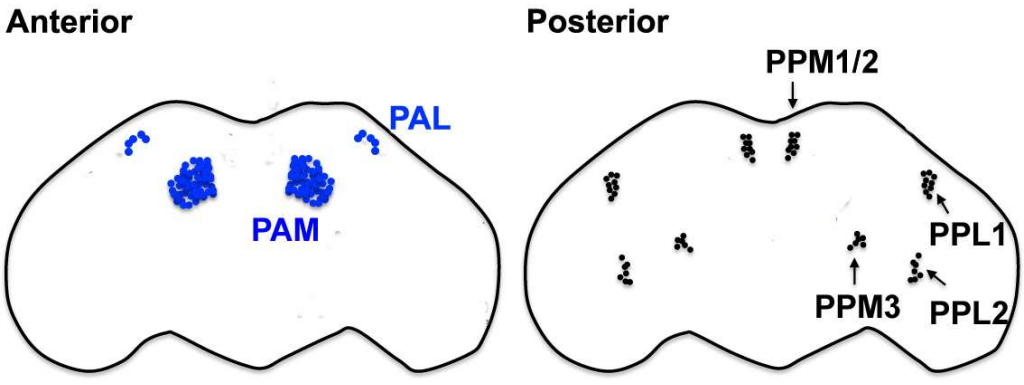

Figure 1. DA neurons in the adult fly brain. Schematics of the seven major clusters of DA neurons located in the anterior and posterior brain [16, 17, 183]. The protocerebral anterior lateral (PAL) and protocerebral anterior medial (PAM) clusters reside in the anterior brain. Major posterior clusters include the protocerebral posterior medial 1, 2 and 3 (PPM1, PPM2 and PPM3), and protocerebral posterior lateral cluster 1 and 2 (PPL1 and PPL2).

A notable limitation of *Drosophila* as a model organism to study PD is the absence of fly homolog of *SNCA,* the gene encoding α-syn. Consequently, Lewy body pathology is not observed endogenously in flies. However, as discussed below, formation of Lewy body-like inclusions can be induced by ectopic expression of human α-syn in flies [20], indicating that cellular machineries necessary for Lewy body formation and pathophysiological impact of inclusions can be studied in flies.

4. Chemically induced models of sporadic PD

4.1. The environmental toxicology of PD

Epidemiological studies have suggested that several environmental factors, including pesticide exposure, rural living, well-water drinking and agricultural occupation, increase risk of PD [24]. Exposure to pesticides is the most consistently found environmental risk factor for PD. A meta-analysis study reported a 62% increase in PD risk, comparing ever versus never pesticide exposure [25]. The pesticide exposure dose-dependently correlates with the risk of PD, which partly explains the increased risk in agricultural workers [26-29]. Although some inconsistency and heterogeneity among association studies have been reported [30, 31], the idea to translate chronic pesticide use into animal models comes naturally. Indeed, the advent of classic strategy to model sporadic PD using compounds found in pesticides dates before the expansion of the genetic models of PD.

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

Pesticides include insecticides, herbicides and fungicides, and encompass over 900 compounds belonging to many different chemical classes [32]. It is therefore tremendously difficult to determine which compounds the pesticide users were exposed to. Consequently, only a small handful of chemicals have been used to develop PD models in animals. Rotenone and paraquat are among the most widely used.

Rotenone is a naturally occurring chemical found in the roots of tropical plants, commonly used as an insecticide. Being plant-derived and organic, it was originally thought to be harmless to humans when properly used. However, epidemiological studies have shown the increased risk for PD in rotenone users [33], and its use has been prohibited or restricted in many countries. It is a lipophilic compound that can freely cross blood brain barriers and cell membrane. Toxicity of rotenone is largely attributed to its effect on the inhibition of mitochondrial complex I activity [34], although other mechanisms, including microtubule depolymerization, may contribute to its toxicity [35].

Although not a common environmental toxin, MPTP (1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridin) was the first chemical that demonstrated the role of neurotoxins in the etiology of "Parkinsonism" (a set of symptoms typically associated with PD). MPTP is a byproduct of the synthesis of a meperidine analog MPPP (1-methyl-4-phenyl-4-propionoxypiperidine), a synthetic opioid drug, and was found to be the causative agent of the symptoms similar to advanced PD in the intravenous drug users [36]. MPTP crosses the blood brain barrier and is converted to the toxic 1-methyl-4-phenylpyridinium ion (MPP^+) by monoamine oxidase B (MAO-B) in glia. Due to is high affinity to the dopamine transporter (DAT), MPP^+ is selectively taken

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

up to DA neurons. Thus, MPTP exposure causes rapid, non-progressive DA neuron loss and associated motor disabilities in humans, as in various animal models [37].

Clinically, MPTP exposure is now one of the exclusion criteria for PD because of the known consequence of this drug's action, that is acute and non-progressive Parkinsonism, unlike in chronic PD [1]. However, the discovery of MPTP boosted a search for its analogs, including paraquat, one of the most widely used herbicides. Paraquat is structurally similar to MPP^+, can cross BBB and is likely to be taken up to DA neurons as a form of $paraquat^+$ [38]. It is an oxidative stressor that, by acting as a redox cycler, produces superoxide radicals and other reactive oxygen species (ROS). Paraquat also increases superoxide radical production in mitochondria [39, 40]. Several case-controlled epidemiological studies have shown the increased risk of PD with paraquat exposure [33, 41].

4.2. The use of rotenone and paraquat to model sporadic PD and its variations

Chronic rotenone exposure is one of the first toxin-based fly models of sporadic PD. Flies fed with sublethal dose of rotenone (< 750 μM) for 7 days show impaired locomotor ability in startle-induced climbing assays and neuronal loss selectively in several subclasses of DA neurons. The administration of Levodopa (L-dopa), the most effective symptomatic medication for PD, partially rescues rotenone-induced climbing defects but not neuronal loss. L-dopa can cross the blood brain barrier, be taken up by DA neurons and converted to DA by dopa decarboxylase. These results altogether indicate that chronic rotenone exposure induces selective loss of DA neurons, leading to the reduced DA signaling and locomotor deficits, and thus recapitulates key characteristics of PD [19]. Using a slightly modified protocol, another group also showed that a 10-day rotenone (250 μM) exposure induces the loss

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

of DA neurons in the PPL1 and PAL clusters, which progresses at least up to 6 weeks after the treatment [42].

Similar to rotenone, paraquat has been frequently used in different model animals to test its causal relationship with the pathological characteristics of PD. Several studies in flies have consistently demonstrated that chronic paraquat exposure causes a DA neuron loss or functional impairments and locomotor deficits within hours to days, accompanied by high lethality. Feeding flies with 20 mM paraquat induces locomotor defects within 12 h and a neuronal loss selectively in several classes of DA neurons as early as 6 h after the treatment. Despite such rapid and drastic effects, it does not induce obvious brain-wide tissue damage, judging from the overall morphology of the brain and cholinergic neurons [43]. Similarly, DA neuron loss in the PPL1 cluster was observed 24 h after the exposure to 10 mM paraquat [42]. In another study, 20 mM paraquat ingestion induced pathological morphological changes within 24 h in several subclasses (PPL2, PPL2, PAL and PP2) of DA neurons, such as shrinkage of cell bodies, indicative of functional impairments [44].

These chemically induced models have successfully shown the relevance of environmental agents, in particular pesticides rotenone and paraquat, in DA neuron demise and motor disabilities, but not without criticism. In general, toxins fail to recapitulate the progressive nature of neurodegeneration in PD, though rotenone-induced progressive DA neuron loss is reported [42]. An inevitable drawback is the lethality (they are pesticides, after all); depending on the concentration and application route, rotenone and paraquat kill flies within hours to days. The dose of rotenone or paraquat effective to induce DA neuron loss falls within the range where median survival time is 7 days or less during the chronic exposure [42]. Furthermore, there are some discrepancies among the results of different studies, which may come

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

from the subtle differences in the toxin administration protocols and laboratory environment. Absence of DA neuron loss after the chronic exposure of 500 μM rotenone or 100 μM paraquat for 7 to 10 days was reported [45]. A study found a morphological sign of declined viability in DA neurons in the flies exposed to 500 μM rotenone but no aberrant changes in DA neurons in the flies treated with 20 mM paraquat for 72 h[46].

Nevertheless, these apparent shortcomings can be considered as evidence supporting the multi-factorial origin of sporadic PD. The inconsistency in the results is indicative of the necessity of synergism with other chemicals or genetic variants for DA neuron demise and locomotor deficits. It is extremely difficult to measure the dose of environmental toxin exposure to humans, but it's unlikely to be comparable to the lethal or sublethal dose in the toxin ingestion experiments in flies. Thus, more realistic models of sporadic PD may be created by combining multiple chemicals of non-effective drug doses or by exposing single or multiple chemicals to the flies of different genetic backgrounds. The synergism of chemical agents and genetic factors can be tested on known candidate genetic variants or by screening for genetic modifiers that enhance or suppress the effect of the chemicals.

In line with this view, a number of studies have examined the synergism of multiple compounds or compounds and genetic factors on PD risk. Maneb (manganese ethylenebisdithiocarbamate) and ziram (zinc dimethylbisdithiocarbamate) are commonly used fungicides that belong to the class of dithiocarbamates. Epidemiological studies evaluating the effect of ambient exposure to pesticides found an increased risk of PD associated with maneb or ziram alone and the greater risk with the combined use of paraquat, maneb and ziram [47, 48]. In flies, chronic exposure to either 4 mM paraquat, 500 μM maneb, or 1mM ziram (the dose that does

Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 3343; doi:10.3390/ijms19113343

not decrease survival) does not individually impair DA neuron viability or climbing ability. In contrast, the combined exposure of paraquat and maneb, but not paraquat and ziram, leads to DA neuron loss in the PPL1 cluster at 6 weeks [42, 49].

The approach to combine toxin exposure and genetic manipulations in flies is increasingly common and has uncovered important molecular insights into the gene-environmental interactions that increase or decrease PD risk. One study showed that RNAi-mediated knockdown of the gene encoding tyrosine hydroxylase (TH), the rate-limiting enzyme for DA synthesis, in several subclasses of DA neurons extends the life span and partially suppresses DA neuron loss in rotenone-treated flies (500 μM chronic exposure). These results suggest that DA production enhances the deleterious effects of rotenone on the viability of DA neurons and life span [50]. Similarly, another study found that overexpression of *Drosophila vesicular monoamine transporter* (*dVMAT*), which is required for packaging DA into synaptic vesicles, protects DA neurons from rotenone-induced loss, presumably by reducing cytosolic DA pool [42]. These findings partly explain why DA neurons are selectively sensitive to rotenone and are concordant with the hypothesis that DA metabolism is a contributing factor in the selective vulnerability of SNpc DA neurons in PD [51].

In contrast, several studies point to the opposite relationship of DA production and toxicity in paraquat models of PD; that is, increase in DA production confers protection against paraquat-induced neurotoxicity. Loss-of-function mutations in the *Catecholamines-up* (*Catsup*) gene encoding a negative regulator of DA production unexpectedly promote survival and protect DA neurons from degeneration in the paraquat-treated flies[43]. Because overexpression of dVMAT, which leads to the reduction of cytosolic DA pool, does not enhance or exacerbate paraquat-induced DA neuron loss [42], the increase in extracellular DA rather than cytosolic DA probably

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

reduces paraquat-induced neurotoxicity. Parallel to these observations was the finding that overexpression of the TH gene in DA neurons or in serotonergic neurons equally promotes resistance against lethality in paraquat-treated flies [44]. This study also asked how the increase in extracellular DA counteracts the paraquat-induced neurotoxicity by correlating the expression of D_1-like dopamine receptor, DAMB, and the toxicity. DA signaling via DAMB normally increases cytosolic Ca^{2+} levels, which, in excess, enhances oxidative stress and excitotoxicity. However, they found that TH overexpression causes a compensatory downregulation of DAMB in postsynaptic glutamatergic neurons, thereby confers resistance against paraquat-induced neurotoxicity [44].

Thus, rotenone-induced neurotoxicity may be chiefly mediated cell-autonomously by the mechanism sensitive to the cytosolic DA levels. In contrast, systemic neurotoxicity of paraquat, including the selective loss of DA neurons, is DA-dependent but mediated by the non cell-autonomous mechanisms. This intriguing mechanistic difference may be attributable to toxin's biochemical mechanisms of action. Both rotenone and paraquat cause oxidative damage but through distinct molecular events. Toxicity of rotenone is mainly caused by the inhibition of mitochondrial complex I [52], whereas paraquat toxicity is thought to be essentially due to its redox cycling, leading to the production of ROS in the form of hydrogen peroxide (H_2O_2) and hydroxyl radicals (HO) and oxidation of NADPH, the major source of reducing equivalents in the cell [53]. H_2O_2 can diffuse across the plasma membrane through aquaporin channels [54]. Therefore, it is possible that rotenone's immediate toxicity is mainly confined within the affected cells, whereas paraquat toxicity is more easily spread among cells.

Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 3343; doi:10.3390/ijms19113343

A recent study by Stephano et al. [55] showed that chronic exposure to 500 µM rotenone does not cause significant neuronal DA neuron loss in their hands at least up to 10 days, whereas climbing ability declines from day 6 onwards. They took advantage of the absence of neuronal loss and isolated DA neurons after 3 days of rotenone treatment, the timepoint considered as the presymptomatic phase, and analyzed their transcriptome. Differential gene expression analysis comparing the DA-neurons of rotenone-treated and control flies revealed the gene expression signature indicating the activation of MAPK/EGFR- and TGF-β signaling pathways and inhibition of the Wnt signaling pathway in rotenone-treated flies, including a reduced expression of *armadillo*/*β-catenin*, a central component of the Wnt signaling. Concordant with this finding, overexpression of *armadillo* in DA neurons ameliorates rotenone-induced locomotor decline, suggesting a role for the Wnt signaling in the pathogenesis of PD [55]. Further implications of this work include the potential use of transcriptomic datasets to identify prodromal disease biomarkers.

Mitochondrial uncoupling protein 2 (UCP2) belongs to a class of anion transporter family present in the inner mitochondrial membrane. UCP2 uncouples mitochondrial electrical transport chain from phosphorylation of ADP to ATP synthesis by dissipating the proton gradient across the mitochondrial inner membrane. Through this process UCP2 attenuates ROS production and protect cells from oxidative damage [56]. Overexpression of human UCP2 (hUCP2) in several subclasses of DA neurons ameliorates the locomotor decline and partially prevents DA neuron loss induced by chronic rotenone exposure [57], likely by counteracting mitochondrial damage induced by rotenone [58]. Although whether endogenous *Drosophila* UCPs are involved in rotenone-induced toxicity remains unaddressed, these findings suggest UCP2 as a potential therapeutic target for PD.

Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 3343; doi:10.3390/ijms19113343

In summary, *Drosophila* offers opportunities to delineate toxin-specific mechanism of action on DA neurodegeneration and can provide new insights into understanding PD of diverse etiological origins. Furthermore, the powerful genetic toolbox of *Drosophila* enables the identification of genetic modifiers of toxin-induced neurodegeneration with relative ease. It is anticipated that the study of synergism between toxins and genetic variants will be increasingly popular in order to validate the role of common genetic variants and their underlying mechanisms in PD susceptibility. The toxin-genetic combined approach might even discover new genes, whose cryptic variations normally have little effect on human health but increase PD risk in certain environmental conditions.

5. Genetic models of sporadic PD

The identification of several causative genes for rare monogenic forms of PD, such as *SNCA* (*α-synuclein*) [3], *Leucine-rich repeat kinase 2* (*LRRK2*) [59], *Parkin* [60], *PTEN-induced putative kinase 1* (*PINK1*) [61] and *DJ-1* [62] overturned the traditional view that PD is not hereditary and unequivocally demonstrated the major role of genetics in the PD etiology [63]. Furthermore, the past decade has seen a considerable progress in identifying genetic risk variants associated with PD through linkage analysis or genome-wide association studies (GWAS). Recent GWAS meta analyses have so far identified 41 loci, encompassing in total of 72 candidate genes, associated with sporadic PD risk [64, 65]. Over 70% of the PD risk loci have either protein altering or cis-QTL variants, suggesting that changes in the function or expression levels of candidate genes are associated with the increased PD risk (Table 1). Importantly, in agreement with previous studies, these studies found polymorphic variants of *SNCA* and *LRRK2*, also known as causal genes of monogenic forms of PD,

Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 3343; doi:10.3390/ijms19113343

in association with sporadic PD. These studies also confirmed *MAPT* and *GBA* as PD-susceptibility factors [66].

Table 1. Candidate genetic risk factors for PD identified in a recent GWAS meta-analysis. The table is adapted from [64]. Genes in bold font are also linked to the monogenic forms of familial PD.

Chromosome	Gene
1	*GBA, NUCKS1, SLC41A1, ITPKB, SIPA1L2*
2	*IL1R2, TMEM163, CCNT2, SCN3A, STK39*
3	*CHMP2B, MCCC1, SATB1, NCKIPSD, CCDC71, ALAS1, TLR9, DNAH1, BAP1, PHF7, NISCH, STAB1, ITIH3, ITIH4*
4	*TMEM175, DGKQ, FAM200B, CD38, FAM47E,* ***SNCA****, ANK2, CAMK2D*
5	*ELOVL7*
6	*ZNF184, HLA–DRB6, HLA–DQA1*
7	*KLHL7, NUPL2, GPNMB*
8	*MICU3, CTSB, SORBS3, PDLIM2, C8orf58, BIN3*
9	*SH3GL2*
10	*FAM171A1, BAG3*
11	*DLG2, MIR4697*
12	***LRRK2****, OGFOD2*
14	*GCH1, TMEM229B, GALC*
15	*VPS13C*
16	*ZNF646, KAT8, COQ7, TOX3*
17	*ARHGAP27, CRHR1, SPPL2C, MAPT, STH, KANSL1, ATP6V0A1, PSMC3IP,*
18	*SYT4*
19	*LSM7*
20	*DDRGK1*

The discovery of genetic factors implicated in PD risk has motivated the generation of genetic models in both vertebrate and invertebrate animals [67, 68]. These models have been instrumental to advance our understanding of pathophysiology and molecular mechanisms of PD. In flies, most common approach to generate genetic models is to introduce causal or disease-associated genetic variations in their fly homologs or by expressing human genes in wild-type or mutant forms. The latter is applicable only for dominant mutations. These methodologies are used for modeling both familial and sporadic PD. In fact, the genetic models based on

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

the familial PD-linked genes that are also implicated in sporadic PD (i.e., *SNCA* and *LRRK2*) are often not specifically designated as sporadic PD models. This is in line with the view that the boundaries between familial and sporadic PD are increasingly blurred. The following sections will first discuss the targeted genetic models based on familial PD-causal genes, whose common variants are associated with sporadic PD risk. Secondly, the models developed based on the genetic susceptibility factors associated with PD will be introduced. Lastly, the genetic models of sporadic PD developed through serendipity will be discussed.

5.1. Familial PD-linked genes in sporadic PD: SNCA and LRRK2

One of the most notable discoveries with regard to the genetics of sporadic PD is that some low-penetrance variants of the familial PD-linked genes, *SNCA* and *LRRK2*, are also present in sporadic cases [7, 66, 69]. *SNCA*, encoding α-syn, is a causal gene of autosomal dominant familial PD. Three pathogenic mutations (A30P, E46K and A53T) as well as gene duplications and triplications have been identified in approximately 1% of PD families [70, 71]. *SNCA* dominant mutations are extremely rare; however, the discovery that α-syn is the major constituent of the Lewy bodies [3], the pathological hallmark of most familial and sporadic PD, prompted the search for *SNCA* polymorphisms in sporadic cases. Indeed, duplications have been found in apparently sporadic cases [72, 73] and also in asymptomatic carriers [72, 74], with the estimated penetrance of approximately 40% [72, 75]. These findings suggest the presence of other genetic modifiers or environmental factors in the pathogenesis of PD due to SNCA duplication. Association of PD risk with polymorphisms in the promoter region and 3' UTR of the SNCA gene is also reported [76-78]. The

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

duplications and variants likely increase the expression levels of α-syn protein, thereby contributing to the PD pathogenesis [79-81].

α-Syn is abundantly expressed in the nervous system and enriched in the presynaptic terminals [82]. It is highly soluble and unfolded in aqueous solutions but can self-assemble to form fibrils. Multiple lines of evidence suggest that soluble prefibrillar form of α-syn oligomers, rather than larger aggregates, are the toxic species mediating the cellular dysfunction and degeneration [83]. α-Syn toxicity is rooted in its propensity for oligomerization and disease-causing mutants are more prone to oligomerization than wild-type α-Syn [83]. Recent studies have also shown that α-syn oligomers can be released from neurons and spread in a prion-like manner by seeding with the soluble α-syn of the recipient cells [84-87].

Although *Drosophila* have no *SNCA* homologs, because genetic and molecular evidence indicates that toxic gain-of-function mechanisms underlie α-syn-linked PD, transgenic overexpression of human α-syn in flies provides useful tools to study the pathogenesis of α-syn-linked PD. The overexpression of human wild-type or mutant form of α-syn was indeed the first PD model ever created in flies [20]. Similar strategies have been used in a number of studies ever since and shown that overexpression of wild-type or mutant human α-syn impairs the function or survival of DA neurons and causes PD-like locomotor deficits [20, 22, 88-91], although some discrepancies exist regarding its effect on DA neuron loss [46, 92, 93].

Mutations in *LRRK2* are the most frequent cause of PD, found in approximately 10% of autosomal dominant familial PD and 4% of sporadic cases world-wide[94]. The most common mutation, G2019S, is found in 4% of familial and 1% of apparently sporadic cases. Estimated penetrance of G2019S mutation ranges from approximately 30 to 70%, depending on the age and ethnic background [95].

Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 3343; doi:10.3390/ijms19113343

G2019S mutation is especially frequent in sporadic PD patients among Ashkenazi Jews (13%) [96] and North African Arabs (41%) [97]. No disease case caused by *LRRK2* deletion or multiplications have been reported, and disease phenotypes of homozygous LRRK2 mutant carriers are similar to those of heterozygous carriers [98]. Altogether, these findings suggest that LRRK2 G2019S manifests PD by gain-of-function mechanisms involving the interaction with other genetic or environmental factors.

LRRK2 encodes a large, 2527 amino-acid protein containing multiple conserved domains, including a GTPase domain, a kinase domain, Armadillo, Ankyrin repeat, leucine rich repeat, MAPKKK (MAP kinase kinase kinase) and WD-40 domains [99]. G2019S mutation resides in the MAPKKK domain and the mutation increases the kinase activity by two- to three-fold at least *in vitro* [100, 101]. *Drosophila LRRK* (*dLRRK*) is the unique homolog of human *LRRK2*. Consistent with the notion that LRRK2 gain-of-function mutations cause PD, *dLRRK2* null mutant flies show little or no defects in the viability of DA neurons [102], although some controversy exists [103]. A number of transgenic fly lines expressing wild-type or mutant form of dLRRK or human LRRK2 have been generated in an attempt to model dominantly inherited PD, with varying consequences in neuronal loss and locomotion. Pan-neuronal or DA neuron-targeted expression of human LRRK2 G2019S mutants, the genetic model relevant for sporadic PD, causes selective age-dependent loss of DA neurons, locomotor deficits, and premature mortality [104-108].

Since there are many excellent reviews summarizing the findings on α-syn or LRRK2 models in flies (such as[109-112]), instead of listing up findings from the individual papers, this review elects to briefly discuss overarching insights from a large body of work on these models. One of the emerging notions is the diversity of

Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 3343; doi:10.3390/ijms19113343

the mechanisms underlying the DA neuron demise. There is so far no single, unified theory explaining the pathogenic mechanism of α-syn overexpression or LRRK2 G2019S mutation, but various intracellular machineries are implicated. Studies using *Drosophila* models have shown that the alterations in ER-Golgi trafficking and synaptic vesicle formation [113, 114], glutathione abundance [91], glucose metabolism [115], actin cytoskeleton that leads to impaired mitochondrial dynamics [90], and elevated oxidative stress levels [116] are involved in the α-syn pathogenicity. A growing number of potential LRRK2 phosphorylation targets involved in PD pathogenesis has been identified in the studies using the fly model of *LRRK2*-linked PD, including the glycogen synthase kinase 3b (GSK 3b) [105], microtubule-binding protein 1B (MAP1B) homolog Futsch [117], endophilin A (EndoA)[118, 119], eukaryotic initiation factor 4E-binding protein (4E-BP) [104] and ribosome protein s15 [120]. The phosphorylation of these targets by LRRK2 dysregulate cellular processes such as dendritic and synaptic morphology, synaptic endocytosis, macroautophagy, and translational machinery. In addition, genetic and functional interaction assays found several factors involved in the neurotoxicity of LRRK2, such as vacuolar protein sorting 35 (VPS35) [121, 122], RAB7L1 [123], ArfGAP1 [124, 125], which are involved in vesicular trafficking, retromer, lysosomal pathway and miRNA-mediated translational inhibition.

The discovery of these numerous targets and pathways leads us to the next important questions: how are the multiple pathways related? Are they additively or synergistically act to disrupt cellular homeostasis, thereby leading to the eventual cell death? Given the clinical, geographic and ethnic heterogeneity of PD patients, it is also possible that different pathogenic mechanisms are triggered depending on the genetic background, environmental conditions or in a state-dependent manner. There

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

is so far no clear view as to why the same mutation (e.g. *LRRK2* G2019S) is causal to both familial and sporadic PD. Understanding whether and how different pathogenic mechanisms interact will help comprehend the mechanistic differences between familial versus sporadic PD, for which *Drosophila* models can provide useful tools.

Both α-syn and LRRK G2019S models are based on the overexpression by the use of binary systems, such as GAL4/UAS and LexA/LexAop [14]. Although the results obtained by the misexpression of foreign genes have to be interpreted with a grain of salt, the carefully controlled misexpression experiments can be useful to analyze cell-type specificity of toxicity, neuroanatomical correlate with behavioral phenotype and to distinguish cell-autonomous from non-cell-autonomous consequences of overexpressed genes. A prime example is the use of α-syn overexpression to map the neuronal subtypes relevant for climbing defects, which identified a subgroup of DA neurons within the PAM cluster, suggesting functional parallels between this group of neurons with DA neurons in the SNpc [22]. Another example is the overexpression of LRRK2 G2019S in DA neurons, which leads to non-cell-autonomous degeneration in the visual system and loss of visual function [126]. With the use of advanced genetic tools that enable the gene expression in spatially and temporarily tunable fashion, it is likely that misexpression strategy will provide further insights into the mechanisms underlying PD associated with gain-of-function mutations.

5.2. Models based on known genetic risk factors: GBA, MAPT and GAK

5.2.1. GBA

Homozygous or compound heterozygous mutations in the *GBA* gene encoding β-glucocerebrosidase (GCase) cause Gaucher disease. Gaucher disease is a rare lipid storage disorder, and the patients are known to have increased risk for PD.

Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 3343; doi:10.3390/ijms19113343

Additionally, single heterozygous mutations are associated with the increased risk of PD [7, 64, 66]. Approximately 300 mutations in *GBA* have been found to cause Gaucher diseases. Including the two common mutations associated with PD, N370S and L444P, *GBA* mutations are considered to be loss-of-function mutations leading to the deficiency in GCase. GCase is a lysosomal enzyme synthesized in the endoplasmic reticulum (ER), undergoes N-linked glycosylation and folded, and subsequently traffics to lysosomes through the Golgi. GBA mutations results in the accumulation of lipid substrates of GCase [127]. Additionally, accumulation of misfolded mutant GCase causes ER stress and triggers the unfolded protein response (UPR). Resulting accumulation of misfolded protein, including that of α-syn, is thought to increase the risk of PD [128, 129].

Drosophila has two homologs of the *GBA* gene, *dGBA1a* and *dGBA1b*, both of which have approximately 50% similarity to GCase. Since disease causing GBA variants are loss-of-function alleles, several *dGBA1a/b* gene knockout or knockdown models that inactivate either or both of the *dGBA1* genes have been generated [130-133]. In addition, the finding that many mutations including PD-associated N370S and L444P are likely to be dominant-negative led to the creation of transgenic flies expressing wild-type of mutant human *GBA* [129, 134, 135] . The phenotypes of these loss-of-function and misexpression models are roughly in agreement that they show reduced lifespan, locomotor impairments, protein aggregation and neurodegeneration.

By contrast, the results are less consistent with regard to DA neuron loss in the *dGBA1* models. Some reported the loss of adult DA neurons [132, 134, 135], whereas others showed no DA neuron loss although brain vacuolization was observed in *dGBA1* loss-of-function models [130]. One study reported that, in the flies expressing α-syn, *dGBA1b* mutation increases the misfolding of α-syn and mildly enhances α-

syn-dependent DA neuron loss but other phenotypes of *dGBA1b* mutation are unaffected by the presence of α-syn. Therefore, DA neuron degeneration is primarily the consequence of α-syn overexpression and α-Syn and dGBA1 do not synergistically impair neuronal integrity [130].

Although these differences warrant further studies to clarify the contribution of dGBA1 in DA neuron degeneration and its underlying mechanisms, fly models have been effectively used to demonstrated that small molecule pharmacological chaperones that cross the blood brain-barrier, ambroxol and isofagomine, suppress GCase misfolding, reduce ER stress and ameliorate the GBA mutant phenotype *in vivo*. These results provide supporting evidence that ER stress is a pathogenic process in GBA mutants and suggest a therapeutic potential of small molecule chaperons in GBA-associated PD [132, 134]. Collectively, *dGBA1* mutation and hGBA misexpression models provide useful tools to better understand the molecular mechanisms underlying the pathogenic process caused by GBA mutations and developing disease-modifying therapeutics.

5.2.2. MAPT

The *MAPT* gene encodes the microtubule-associated protein tau essential for tubulin polymerization and microtubule stability [136]. Accumulation of misfolded tau protein is a pathological characteristic of a spectrum of neurological disorders called tauopathies, including Alzheimer's disease, progressive supranuclear palsy, corticobasal degeneration and frontotemporal dementia. Although the majority of tauopathies occur sporadically, more than 50 pathogenic *MAPT* mutations were identified in about 150 families with tauopathies[137]. Tauopathies associated with *MAPT* mutations include familial and sporadic frontotemporal dementia (FTD)[138,

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

139] (also known as "frontotemporal lobar degeneration with MAPT mutation (FTLD-tau) ")[140]. Intriguingly, parkinsonism is a common comorbidity in patients of familial and sporadic tauopathies, and more than 50% of patients of Alzheimer's disease manifest Lewy body pathology [141]. Furthermore, a number of studies including GWAS meta-analyses have confirmed the association of the *MAPT* locus and increased PD risk in Caucasian populations [64, 142]. These findings support the emerging view that tauopathies and synucleinopathies (associated with α-syn inclusions), the two disease classes characterized by the accumulation of pathological protein aggregates, belong to a continuum of disorders with significantly overlapping clinical features and pathogenic mechanisms [143].

The *MAPT* locus lies within a genomic region that has two conserved haplotypes, H1 and H2, defined by numerous single nucleotide polymorphisms [144]. H1 and H2 haplotypes cover the entire *MAPT* gene, differ in orientation and do not recombine [145] as they originate from a 900 kb inversion [146]. The H1 haplotype of the *MAPT* gene is associated with the risk of PD [147, 148] as well as in sporadic tauopathies [149]. It has been shown that H1 haplotype has a greater expression of tau protein than H2 [150, 151] and H2 haplotype has a protective effect in neurodegeneration [152].

Drosophila tau (*dtau*) is a unique homolog of the *MAPT* gene. Given the central role of tau in tauopathies, it is not surprising that much of the related work on fly is based on the overexpression of *Drosophila* or human tau protein in wilt-type or mutant forms to study toxicity of tau inclusions and its underlying mechanisms at cellular and organismal levels (reviewed in [153, 154]). Deletion of *tau* gene is neither detrimental by itself , nor increases the toxicity of human amyloid-β on fly's life span [155]. One study demonstrated that, by using the strategy of ectopically

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

expressing human tau and α-syn in the retina, DA neurons and larval neuromuscular junction, tau overexpression enhances α-syn-mediated DA neuron loss. Conversely, α-syn overexpression enhances tau-induced motor dysfunction, microtubule disorganization and axonal transport defects, among other neuronal dysfunctions [156]. These results are in agreement with the existence of the interaction between tauopathy and synucleinopathy and provide insights into the mechanism underlying the role of tau in PD pathogenesis. Because the H1 haplotype is very polymorphic that includes subhaplotypes, defining specific genetic variants that associate with the increased risk of PD remains to be a major challenge [157, 158]. Thus, fly models based on the polymorphisms in the *MAPT* gene associated with PD risk, rather than overexpression, are yet to be generated.

5.2.3. GAK

A chromosomal locus containing the *GAK* gene has been repeatedly found in GWAS [142, 159]. Among many other genetic risk factors identified in GWAS, association of *GAK* with PD risk may depend on ethnic background and is replicated in mainly Caucasian populations [64, 160]. *GAK* encodes cyclin-G-associated kinase, the association partner of cyclin-G and CDK5, and is involved in diverse cellular mechanisms, including clathrin-mediated endocytosis and mitotic progression [161]. PD-associated GAK gene polymorphism reduces the GAK protein levels and correlates with the increase in α-syn expression [162]. A recent work demonstrated that RNAi-mediated silencing of *auxilin* (*aux*), the fly homolog of *GAK*, in DA neurons leads to age-dependent locomotor impairments, reduced lifespan and progressive neuronal loss in several subclasses of DA neurons. Moreover, *aux* RNAi accelerate the onset and progression of DA neuron loss caused by α-syn

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

overexpression [163]. These results support that *GAK* is a PD risk factor and *aux* gene silencing in flies provide a valid model to study the function of GAK in PD pathogenesis, including the possible synergism with α-syn toxicity.

5.3. Serendipitous fly models of sporadic PD: Fer2 and dfoxo mutants

There exist a few fly models generated by serendipity and that provide tools to study sporadic PD. This may seem an unusual path to model a human disease, as compared to the targeted approach using the knowledge on genetic and chemical factors relevant for the disease. However, historically many animal models have been developed by serendipity, typically through the careful phenotypic analysis of mutant animals carrying spontaneous mutations or induced by forward genetics, and have contributed to the disease study [164]. This approach has the potential to fill the knowledge gap in the genetic factors involved in the disease pathogenesis. Indeed, by using a statistical model termed genome-wide complex trait analysis (GCTA) that quantify "missing heritability" in PD, it was estimated that genetic factors could explain up to 27% of the PD cases. In contrast, only 3-5% have been attributed to the single nucleotide polymorphisms (SNPs) identified in GWAS studies [165]. This finding suggests that many more genetic variants that influence PD risk and could represent novel therapeutic targets may yet to be discovered. The bottom-up approach going from mutant phenotypic characterization to gene discovery has the potential to open a new avenue in PD research.

The bHLH-transcription factor *Fer2* (p48-related 2) in *Drosophila* shares high homology with mammalian transcription factors *p48/ptf1a* and *Ferd3l* (*Nato3*) within the bHLH domain. Both *p48/ptf1a* and *Ferd3l* are involved in the control of neuronal development [166-168]. In the fly brain *Fer2* is expressed in a limited number of

Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 3343; doi:10.3390/ijms19113343

neurons, including a subgroup of circadian pacemaker neurons [169-171] and two subclasses of DA neurons, PAM and PAL [18, 172]. Intriguingly hypomorphic mutants of *Fer2* exhibit PD-like phenotypes typical for fly models of PD. These include locomotor deficits that can be improved by L-dopa administration, reduced lifespan and a progressive DA neuron loss in the PAM cluster. Because fewer numbers of PAM neurons are born in both strong and milder hypomorphic mutants of *Fer2*, the role of *Fer2* in PAM neuron development is indisputable. However, knockdown of *Fer2* restricted in adulthood demonstrated that *Fer2* plays an essential role in the survival of adult DA neurons independent of its role in development. Furthermore, it was also shown that oxidative stress elicited by the oxidants H_2O_2 or paraquat induces DA neuron loss specifically in the PAM neurons in *Fer2* mutant flies. At the cellular levels, *Fer2* mutation causes abnormality in the mitochondrial morphology and autophagosome formation, the well-known pathogenic molecular events implicated in PD, suggesting that *Fer2* controls the expression of genes involved in multiple mechanisms that are normally protective of DA neurons [18, 173]. Collectively, these results suggest that *Fer2* mutant flies are useful for investigating mechanisms of DA neuron demise, especially the gene-environmental interaction with the presence of oxidative stress, thereby providing an opportunity to study pathogenic processes in PD.

FOXO belongs to the family of forkhead box containing transcription factors, known to be the regulator of diverse biological pathways including stress response, metabolism and cellular homeostasis and implicated in numerous diseases [174]. *Drosophila* has a unique homolog of FOXO, encoded by the *dfoxo* gene. Although no direct association of polymorphisms in FOXO factors with PD risk has been identified in human population, changes in gene expression levels in PD patients [175], studies

Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 3343; doi:10.3390/ijms19113343

in rodents and flies suggest a role of FOXO factors in survival of DA neurons in aged animals and in PD models [176-182]. In addition, it was recently found that *dfoxo* null mutants and *Fer2* mutants are phenotypically similar, showing the selective loss of PAM cluster DA neurons, locomotor deficit and reduced life span. Both *Fer2* and *dfoxo* mutants exhibit mitochondrial damage and autophagy defect specifically in PAM neurons. Genetic interaction analysis showed that loss of function of *Fer2* and *dfoxo* additively but not synergistically aggravate the DA neuron loss. Consistent with the finding that mitochondrial and autophagy are commonly affected in both mutants, overexpression of one can rescue the DA neuron loss caused by the mutation of the other. Interestingly, however, unlike in *Fer2* mutants, oxidative stress does not enhance the DA neuron loss in *dfoxo* mutants. These results suggest that the activity of *Fer2* and *dfoxo* are regulated by different cellular stressors but their downstream pathways converge onto the process leading to mitochondrial biology and autophagy, thereby promoting the survival of DA neurons in the PAM cluster [173].

The results of *Fer2* and *dfoxo* mutants highlight the complex interaction between multiple genetic and environmental factors affecting DA neuron viability and its impact on the pathogenesis of PD. It will be of great interest to know whether their homologs, downstream or interacting genes in humans have a causal role in PD pathogenesis.

6. Conclusion

A growing body of work to model sporadic PD in flies has verified candidate environmental and genetic risk factors and provided insights into their roles in the pathogenesis of PD. The current list of genetic risk variants is by no means exhaustive to explain all the sporadic PD cases and, as the expansion and technological

improvement of GWAS continues, many more susceptibility loci are expected to be found and prompt the generation of genetic animal models of sporadic PD in coming years. At the same time, more unbiased approach going from phenotype to genotype, including forward genetic screens, remain to be valuable to identify novel genetic factors modifying the risk or severity of PD. Fly models continue to offer effective tools to better understand whether and how different molecular pathways involved in the degenerative process interplay – the knowledge necessary to develop efficient and possibly personalized treatment strategy for this complex disorder.

Acknowledgements

This work is supported by the research grants from the European Research Council (ERC-2012-StG_20111109, Project number 311194) and the Swiss National Science Foundation (31003A_169548).

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

References

1. Kalia LV, Lang AE. Parkinson's disease. Lancet. 2015;386(9996):896-912.
2. Poewe W. Non-motor symptoms in Parkinson's disease. Eur J Neurol. 2008;15 Suppl 1:14-20.
3. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature. 1997;388(6645):839-40.
4. Kalia LV, Lang AE, Hazrati LN, Fujioka S, Wszolek ZK, Dickson DW, et al. Clinical correlations with Lewy body pathology in LRRK2-related Parkinson disease. JAMA Neurol. 2015;72(1):100-5.
5. Poulopoulos M, Levy OA, Alcalay RN. The neuropathology of genetic Parkinson's disease. Mov Disord. 2012;27(7):831-42.
6. Reeve A, Simcox E, Turnbull D. Ageing and Parkinson's disease: why is advancing age the biggest risk factor? Ageing Res Rev. 2014;14:19-30.
7. Schulte C, Gasser T. Genetic basis of Parkinson's disease: inheritance, penetrance, and expression. Appl Clin Genet. 2011;4:67-80.
8. Gasser T. Mendelian forms of Parkinson's disease. Biochim Biophys Acta. 2009;1792(7):587-96.
9. Hernandez DG, Reed X, Singleton AB. Genetics in Parkinson disease: Mendelian versus non-Mendelian inheritance. J Neurochem. 2016;139 Suppl 1:59-74.
10. Baba Y, Markopoulou K, Putzke JD, Whaley NR, Farrer MJ, Wszolek ZK, et al. Phenotypic commonalities in familial and sporadic Parkinson disease. Arch Neurol. 2006;63(4):579-83.
11. Papapetropoulos S, Adi N, Ellul J, Argyriou AA, Chroni E. A prospective study of familial versus sporadic Parkinson's disease. Neurodegener Dis. 2007;4(6):424-7.
12. McGonigle P, Ruggeri B. Animal models of human disease: challenges in enabling translation. Biochem Pharmacol. 2014;87(1):162-71.
13. Pandey UB, Nichols CD. Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. Pharmacol Rev. 2011;63(2):411-36.
14. Venken KJ, Simpson JH, Bellen HJ. Genetic manipulation of genes and cells in the nervous system of the fruit fly. Neuron. 2011;72(2):202-30.
15. Fernandez-Hernandez I, Scheenaard E, Pollarolo G, Gonzalez C. The translational relevance of Drosophila in drug discovery. EMBO Rep. 2016;17(4):471-2.
16. Mao Z, Davis RL. Eight different types of dopaminergic neurons innervate the Drosophila mushroom body neuropil: anatomical and physiological heterogeneity. Front Neural Circuits. 2009;3:5.
17. Yamamoto S, Seto ES. Dopamine dynamics and signaling in Drosophila: an overview of genes, drugs and behavioral paradigms. Exp Anim. 2014;63(2):107-19.
18. Bou Dib P, Gnagi B, Daly F, Sabado V, Tas D, Glauser DA, et al. A conserved role for p48 homologs in protecting dopaminergic neurons from oxidative stress. PLoS Genet. 2014;10(10):e1004718.
19. Coulom H, Birman S. Chronic exposure to rotenone models sporadic Parkinson's disease in Drosophila melanogaster. J Neurosci. 2004;24(48):10993-8.

20. Feany MB, Bender WW. A Drosophila model of Parkinson's disease. Nature. 2000;404(6776):394-8.
21. Nichols CD, Becnel J, Pandey UB. Methods to assay Drosophila behavior. J Vis Exp. 2012(61).
22. Riemensperger T, Issa AR, Pech U, Coulom H, Nguyen MV, Cassar M, et al. A single dopamine pathway underlies progressive locomotor deficits in a Drosophila model of Parkinson disease. Cell Rep. 2013;5(4):952-60.
23. Sun J, Xu AQ, Giraud J, Poppinga H, Riemensperger T, Fiala A, et al. Neural Control of Startle-Induced Locomotion by the Mushroom Bodies and Associated Neurons in Drosophila. Front Syst Neurosci. 2018;12:6.
24. Noyce AJ, Bestwick JP, Silveira-Moriyama L, Hawkes CH, Giovannoni G, Lees AJ, et al. Meta-analysis of early nonmotor features and risk factors for Parkinson disease. Ann Neurol. 2012;72(6):893-901.
25. van der Mark M, Brouwer M, Kromhout H, Nijssen P, Huss A, Vermeulen R. Is pesticide use related to Parkinson disease? Some clues to heterogeneity in study results. Environ Health Perspect. 2012;120(3):340-7.
26. Ascherio A, Chen H, Weisskopf MG, O'Reilly E, McCullough ML, Calle EE, et al. Pesticide exposure and risk for Parkinson's disease. Ann Neurol. 2006;60(2):197-203.
27. Dick FD, De Palma G, Ahmadi A, Scott NW, Prescott GJ, Bennett J, et al. Environmental risk factors for Parkinson's disease and parkinsonism: the Geoparkinson study. Occup Environ Med. 2007;64(10):666-72.
28. Elbaz A, Clavel J, Rathouz PJ, Moisan F, Galanaud JP, Delemotte B, et al. Professional exposure to pesticides and Parkinson disease. Ann Neurol. 2009;66(4):494-504.
29. Van Maele-Fabry G, Hoet P, Vilain F, Lison D. Occupational exposure to pesticides and Parkinson's disease: a systematic review and meta-analysis of cohort studies. Environ Int. 2012;46:30-43.
30. Bellou V, Belbasis L, Tzoulaki I, Evangelou E, Ioannidis JP. Environmental risk factors and Parkinson's disease: An umbrella review of meta-analyses. Parkinsonism Relat Disord. 2016;23:1-9.
31. Breckenridge CB, Berry C, Chang ET, Sielken RL, Jr., Mandel JS. Association between Parkinson's Disease and Cigarette Smoking, Rural Living, Well-Water Consumption, Farming and Pesticide Use: Systematic Review and Meta-Analysis. PLoS One. 2016;11(4):e0151841.
32. Casida JE. The greening of pesticide-environment interactions: some personal observations. Environ Health Perspect. 2012;120(4):487-93.
33. Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, et al. Rotenone, paraquat, and Parkinson's disease. Environ Health Perspect. 2011;119(6):866-72.
34. Sherer TB, Richardson JR, Testa CM, Seo BB, Panov AV, Yagi T, et al. Mechanism of toxicity of pesticides acting at complex I: relevance to environmental etiologies of Parkinson's disease. J Neurochem. 2007;100(6):1469-79.
35. Choi WS, Kruse SE, Palmiter RD, Xia Z. Mitochondrial complex I inhibition is not required for dopaminergic neuron death induced by rotenone, MPP+, or paraquat. Proc Natl Acad Sci U S A. 2008;105(39):15136-41.
36. Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science. 1983;219(4587):979-80.

37. Meredith GE, Rademacher DJ. MPTP mouse models of Parkinson's disease: an update. J Parkinsons Dis. 2011;1(1):19-33.
38. Rappold PM, Cui M, Chesser AS, Tibbett J, Grima JC, Duan L, et al. Paraquat neurotoxicity is mediated by the dopamine transporter and organic cation transporter-3. Proc Natl Acad Sci U S A. 2011;108(51):20766-71.
39. Cocheme HM, Murphy MP. Complex I is the major site of mitochondrial superoxide production by paraquat. J Biol Chem. 2008;283(4):1786-98.
40. Hosamani R, Muralidhara. Acute exposure of Drosophila melanogaster to paraquat causes oxidative stress and mitochondrial dysfunction. Arch Insect Biochem Physiol. 2013;83(1):25-40.
41. Kamel F, Tanner C, Umbach D, Hoppin J, Alavanja M, Blair A, et al. Pesticide exposure and self-reported Parkinson's disease in the agricultural health study. Am J Epidemiol. 2007;165(4):364-74.
42. Lawal HO, Chang HY, Terrell AN, Brooks ES, Pulido D, Simon AF, et al. The Drosophila vesicular monoamine transporter reduces pesticide-induced loss of dopaminergic neurons. Neurobiol Dis. 2010;40(1):102-12.
43. Chaudhuri A, Bowling K, Funderburk C, Lawal H, Inamdar A, Wang Z, et al. Interaction of genetic and environmental factors in a Drosophila parkinsonism model. J Neurosci. 2007;27(10):2457-67.
44. Cassar M, Issa AR, Riemensperger T, Petitgas C, Rival T, Coulom H, et al. A dopamine receptor contributes to paraquat-induced neurotoxicity in Drosophila. Hum Mol Genet. 2015;24(1):197-212.
45. Meulener M, Whitworth AJ, Armstrong-Gold CE, Rizzu P, Heutink P, Wes PD, et al. Drosophila DJ-1 mutants are selectively sensitive to environmental toxins associated with Parkinson's disease. Curr Biol. 2005;15(17):1572-7.
46. Navarro JA, Hessner S, Yenisetti SC, Bayersdorfer F, Zhang L, Voigt A, et al. Analysis of dopaminergic neuronal dysfunction in genetic and toxin-induced models of Parkinson's disease in Drosophila. J Neurochem. 2014;131(3):369-82.
47. Costello S, Cockburn M, Bronstein J, Zhang X, Ritz B. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. Am J Epidemiol. 2009;169(8):919-26.
48. Wang A, Costello S, Cockburn M, Zhang X, Bronstein J, Ritz B. Parkinson's disease risk from ambient exposure to pesticides. Eur J Epidemiol. 2011;26(7):547-55.
49. Martin CA, Barajas A, Lawless G, Lawal HO, Assani K, Lumintang YP, et al. Synergistic effects on dopamine cell death in a Drosophila model of chronic toxin exposure. Neurotoxicology. 2014;44:344-51.
50. Bayersdorfer F, Voigt A, Schneuwly S, Botella JA. Dopamine-dependent neurodegeneration in Drosophila models of familial and sporadic Parkinson's disease. Neurobiol Dis. 2010;40(1):113-9.
51. Mosharov EV, Larsen KE, Kanter E, Phillips KA, Wilson K, Schmitz Y, et al. Interplay between cytosolic dopamine, calcium, and alpha-synuclein causes selective death of substantia nigra neurons. Neuron. 2009;62(2):218-29.
52. Sherer TB, Betarbet R, Testa CM, Seo BB, Richardson JR, Kim JH, et al. Mechanism of toxicity in rotenone models of Parkinson's disease. J Neurosci. 2003;23(34):10756-64.
53. Bus JS, Gibson JE. Paraquat: model for oxidant-initiated toxicity. Environ Health Perspect. 1984;55:37-46.

54. Bienert GP, Moller AL, Kristiansen KA, Schulz A, Moller IM, Schjoerring JK, et al. Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J Biol Chem. 2007;282(2):1183-92.
55. Stephano F, Nolte S, Hoffmann J, El-Kholy S, von Frieling J, Bruchhaus I, et al. Impaired Wnt signaling in dopamine containing neurons is associated with pathogenesis in a rotenone triggered Drosophila Parkinson's disease model. Sci Rep. 2018;8(1):2372.
56. Brand MD, Esteves TC. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. Cell Metab. 2005;2(2):85-93.
57. Islam R, Yang L, Sah M, Kannan K, Anamani D, Vijayan C, et al. A neuroprotective role of the human uncoupling protein 2 (hUCP2) in a Drosophila Parkinson's disease model. Neurobiol Dis. 2012;46(1):137-46.
58. Hwang RD, Wiemerslage L, LaBreck CJ, Khan M, Kannan K, Wang X, et al. The neuroprotective effect of human uncoupling protein 2 (hUCP2) requires cAMP-dependent protein kinase in a toxin model of Parkinson's disease. Neurobiol Dis. 2014;69:180-91.
59. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron. 2004;44(4):601-7.
60. Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, et al. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet. 2000;25(3):302-5.
61. Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science. 2004;304(5674):1158-60.
62. Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, et al. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science. 2003;299(5604):256-9.
63. Farrer MJ. Genetics of Parkinson disease: paradigm shifts and future prospects. Nat Rev Genet. 2006;7(4):306-18.
64. Chang D, Nalls MA, Hallgrimsdottir IB, Hunkapiller J, van der Brug M, Cai F, et al. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. Nat Genet. 2017;49(10):1511-6.
65. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. Nat Genet. 2014;46(9):989-93.
66. Lesage S, Brice A. Role of mendelian genes in "sporadic" Parkinson's disease. Parkinsonism Relat Disord. 2012;18 Suppl 1:S66-70.
67. Blesa J, Przedborski S. Parkinson's disease: animal models and dopaminergic cell vulnerability. Front Neuroanat. 2014;8:155.
68. Pienaar IS, Gotz J, Feany MB. Parkinson's disease: insights from non-traditional model organisms. Prog Neurobiol. 2010;92(4):558-71.
69. Lesage S, Brice A. Parkinson's disease: from monogenic forms to genetic susceptibility factors. Hum Mol Genet. 2009;18(R1):R48-59.
70. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science. 1997;276(5321):2045-7.
71. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, et al. alpha-Synuclein locus triplication causes Parkinson's disease. Science. 2003;302(5646):841.

72. Ahn TB, Kim SY, Kim JY, Park SS, Lee DS, Min HJ, et al. alpha-Synuclein gene duplication is present in sporadic Parkinson disease. Neurology. 2008;70(1):43-9.
73. Troiano AR, Cazeneuve C, Le Ber I, Bonnet AM, Lesage S, Brice A. Re: Alpha-synuclein gene duplication is present in sporadic Parkinson disease. Neurology. 2008;71(16):1295; author reply
74. Nishioka K, Ross OA, Ishii K, Kachergus JM, Ishiwata K, Kitagawa M, et al. Expanding the clinical phenotype of SNCA duplication carriers. Mov Disord. 2009;24(12):1811-9.
75. Nishioka K, Hayashi S, Farrer MJ, Singleton AB, Yoshino H, Imai H, et al. Clinical heterogeneity of alpha-synuclein gene duplication in Parkinson's disease. Ann Neurol. 2006;59(2):298-309.
76. Kruger R, Vieira-Saecker AM, Kuhn W, Berg D, Muller T, Kuhnl N, et al. Increased susceptibility to sporadic Parkinson's disease by a certain combined alpha-synuclein/apolipoprotein E genotype. Ann Neurol. 1999;45(5):611-7.
77. Mueller JC, Fuchs J, Hofer A, Zimprich A, Lichtner P, Illig T, et al. Multiple regions of alpha-synuclein are associated with Parkinson's disease. Ann Neurol. 2005;57(4):535-41.
78. Tan EK, Tan C, Shen H, Chai A, Lum SY, Teoh ML, et al. Alpha synuclein promoter and risk of Parkinson's disease: microsatellite and allelic size variability. Neurosci Lett. 2003;336(1):70-2.
79. Chiba-Falek O, Nussbaum RL. Effect of allelic variation at the NACP-Rep1 repeat upstream of the alpha-synuclein gene (SNCA) on transcription in a cell culture luciferase reporter system. Hum Mol Genet. 2001;10(26):3101-9.
80. Eriksen JL, Przedborski S, Petrucelli L. Gene dosage and pathogenesis of Parkinson's disease. Trends Mol Med. 2005;11(3):91-6.
81. McCarthy JJ, Linnertz C, Saucier L, Burke JR, Hulette CM, Welsh-Bohmer KA, et al. The effect of SNCA 3' region on the levels of SNCA-112 splicing variant. Neurogenetics. 2011;12(1):59-64.
82. George JM. The synucleins. Genome Biol. 2002;3(1):REVIEWS3002.
83. Stefanis L. alpha-Synuclein in Parkinson's disease. Cold Spring Harb Perspect Med. 2012;2(2):a009399.
84. Brundin P, Melki R. Prying into the Prion Hypothesis for Parkinson's Disease. J Neurosci. 2017;37(41):9808-18.
85. Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, et al. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. Proc Natl Acad Sci U S A. 2009;106(31):13010-5.
86. Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ, et al. Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science. 2012;338(6109):949-53.
87. Peelaerts W, Bousset L, Van der Perren A, Moskalyuk A, Pulizzi R, Giugliano M, et al. alpha-Synuclein strains cause distinct synucleinopathies after local and systemic administration. Nature. 2015;522(7556):340-4.
88. Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM. Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. Science. 2002;295(5556):865-8.
89. Chen L, Feany MB. Alpha-synuclein phosphorylation controls neurotoxicity and inclusion formation in a Drosophila model of Parkinson disease. Nat Neurosci. 2005;8(5):657-63.

90. Ordonez DG, Lee MK, Feany MB. alpha-synuclein Induces Mitochondrial Dysfunction through Spectrin and the Actin Cytoskeleton. Neuron. 2018;97(1):108-24 e6.
91. Trinh K, Moore K, Wes PD, Muchowski PJ, Dey J, Andrews L, et al. Induction of the phase II detoxification pathway suppresses neuron loss in Drosophila models of Parkinson's disease. J Neurosci. 2008;28(2):465-72.
92. Pesah Y, Burgess H, Middlebrooks B, Ronningen K, Prosser J, Tirunagaru V, et al. Whole-mount analysis reveals normal numbers of dopaminergic neurons following misexpression of alpha-Synuclein in Drosophila. Genesis. 2005;41(4):154-9.
93. Whitworth AJ, Wes PD, Pallanck LJ. Drosophila models pioneer a new approach to drug discovery for Parkinson's disease. Drug Discov Today. 2006;11(3-4):119-26.
94. Berg D, Schweitzer KJ, Leitner P, Zimprich A, Lichtner P, Belcredi P, et al. Type and frequency of mutations in the LRRK2 gene in familial and sporadic Parkinson's disease*. Brain. 2005;128(Pt 12):3000-11.
95. Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. Lancet Neurol. 2008;7(7):583-90.
96. Ozelius LJ, Senthil G, Saunders-Pullman R, Ohmann E, Deligtisch A, Tagliati M, et al. LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. N Engl J Med. 2006;354(4):424-5.
97. Lesage S, Durr A, Tazir M, Lohmann E, Leutenegger AL, Janin S, et al. LRRK2 G2019S as a cause of Parkinson's disease in North African Arabs. N Engl J Med. 2006;354(4):422-3.
98. Ishihara L, Warren L, Gibson R, Amouri R, Lesage S, Durr A, et al. Clinical features of Parkinson disease patients with homozygous leucine-rich repeat kinase 2 G2019S mutations. Arch Neurol. 2006;63(9):1250-4.
99. Cookson MR. The role of leucine-rich repeat kinase 2 (LRRK2) in Parkinson's disease. Nat Rev Neurosci. 2010;11(12):791-7.
100. Liu M, Kang S, Ray S, Jackson J, Zaitsev AD, Gerber SA, et al. Kinetic, mechanistic, and structural modeling studies of truncated wild-type leucine-rich repeat kinase 2 and the G2019S mutant. Biochemistry. 2011;50(43):9399-408.
101. Nichols RJ, Dzamko N, Morrice NA, Campbell DG, Deak M, Ordureau A, et al. 14-3-3 binding to LRRK2 is disrupted by multiple Parkinson's disease-associated mutations and regulates cytoplasmic localization. Biochem J. 2010;430(3):393-404.
102. Wang D, Tang B, Zhao G, Pan Q, Xia K, Bodmer R, et al. Dispensable role of Drosophila ortholog of LRRK2 kinase activity in survival of dopaminergic neurons. Mol Neurodegener. 2008;3:3.
103. Lee SB, Kim W, Lee S, Chung J. Loss of LRRK2/PARK8 induces degeneration of dopaminergic neurons in Drosophila. Biochem Biophys Res Commun. 2007;358(2):534-9.
104. Imai Y, Gehrke S, Wang HQ, Takahashi R, Hasegawa K, Oota E, et al. Phosphorylation of 4E-BP by LRRK2 affects the maintenance of dopaminergic neurons in Drosophila. EMBO J. 2008;27(18):2432-43.
105. Lin CH, Tsai PI, Wu RM, Chien CT. LRRK2 G2019S mutation induces dendrite degeneration through mislocalization and phosphorylation of tau by recruiting autoactivated GSK3ss. J Neurosci. 2010;30(39):13138-49.

106. Liu Z, Wang X, Yu Y, Li X, Wang T, Jiang H, et al. A Drosophila model for LRRK2-linked parkinsonism. Proc Natl Acad Sci U S A. 2008;105(7):2693-8.
107. Ng CH, Mok SZ, Koh C, Ouyang X, Fivaz ML, Tan EK, et al. Parkin protects against LRRK2 G2019S mutant-induced dopaminergic neurodegeneration in Drosophila. J Neurosci. 2009;29(36):11257-62.
108. Venderova K, Kabbach G, Abdel-Messih E, Zhang Y, Parks RJ, Imai Y, et al. Leucine-Rich Repeat Kinase 2 interacts with Parkin, DJ-1 and PINK-1 in a Drosophila melanogaster model of Parkinson's disease. Hum Mol Genet. 2009;18(22):4390-404.
109. Li T, Yang D, Sushchky S, Liu Z, Smith WW. Models for LRRK2-Linked Parkinsonism. Parkinsons Dis. 2011;2011:942412.
110. Mizuno H, Fujikake N, Wada K, Nagai Y. alpha-Synuclein Transgenic Drosophila As a Model of Parkinson's Disease and Related Synucleinopathies. Parkinsons Dis. 2010;2011:212706.
111. Whitworth AJ. Drosophila models of Parkinson's disease. Adv Genet. 2011;73:1-50.
112. Xiong Y, Yu J. Modeling Parkinson's Disease in Drosophila: What Have We Learned for Dominant Traits? Front Neurol. 2018;9:228.
113. Breda C, Nugent ML, Estranero JG, Kyriacou CP, Outeiro TF, Steinert JR, et al. Rab11 modulates alpha-synuclein-mediated defects in synaptic transmission and behaviour. Hum Mol Genet. 2015;24(4):1077-91.
114. Cooper AA, Gitler AD, Cashikar A, Haynes CM, Hill KJ, Bhullar B, et al. Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science. 2006;313(5785):324-8.
115. Knight AL, Yan X, Hamamichi S, Ajjuri RR, Mazzulli JR, Zhang MW, et al. The glycolytic enzyme, GPI, is a functionally conserved modifier of dopaminergic neurodegeneration in Parkinson's models. Cell Metab. 2014;20(1):145-57.
116. Botella JA, Bayersdorfer F, Schneuwly S. Superoxide dismutase overexpression protects dopaminergic neurons in a Drosophila model of Parkinson's disease. Neurobiol Dis. 2008;30(1):65-73.
117. Lee S, Liu HP, Lin WY, Guo H, Lu B. LRRK2 kinase regulates synaptic morphology through distinct substrates at the presynaptic and postsynaptic compartments of the Drosophila neuromuscular junction. J Neurosci. 2010;30(50):16959-69.
118. Matta S, Van Kolen K, da Cunha R, van den Bogaart G, Mandemakers W, Miskiewicz K, et al. LRRK2 controls an EndoA phosphorylation cycle in synaptic endocytosis. Neuron. 2012;75(6):1008-21.
119. Soukup SF, Kuenen S, Vanhauwaert R, Manetsberger J, Hernandez-Diaz S, Swerts J, et al. A LRRK2-Dependent EndophilinA Phosphoswitch Is Critical for Macroautophagy at Presynaptic Terminals. Neuron. 2016;92(4):829-44.
120. Martin I, Kim JW, Lee BD, Kang HC, Xu JC, Jia H, et al. Ribosomal protein s15 phosphorylation mediates LRRK2 neurodegeneration in Parkinson's disease. Cell. 2014;157(2):472-85.
121. Inoshita T, Arano T, Hosaka Y, Meng H, Umezaki Y, Kosugi S, et al. Vps35 in cooperation with LRRK2 regulates synaptic vesicle endocytosis through the endosomal pathway in Drosophila. Hum Mol Genet. 2017;26(15):2933-48.
122. Linhart R, Wong SA, Cao J, Tran M, Huynh A, Ardrey C, et al. Vacuolar protein sorting 35 (Vps35) rescues locomotor deficits and shortened lifespan in

Drosophila expressing a Parkinson's disease mutant of Leucine-Rich Repeat Kinase 2 (LRRK2). Mol Neurodegener. 2014;9:23.
123. MacLeod DA, Rhinn H, Kuwahara T, Zolin A, Di Paolo G, McCabe BD, et al. RAB7L1 interacts with LRRK2 to modify intraneuronal protein sorting and Parkinson's disease risk. Neuron. 2013;77(3):425-39.
124. Gehrke S, Imai Y, Sokol N, Lu B. Pathogenic LRRK2 negatively regulates microRNA-mediated translational repression. Nature. 2010;466(7306):637-41.
125. Xiong Y, Yuan C, Chen R, Dawson TM, Dawson VL. ArfGAP1 is a GTPase activating protein for LRRK2: reciprocal regulation of ArfGAP1 by LRRK2. J Neurosci. 2012;32(11):3877-86.
126. Hindle S, Afsari F, Stark M, Middleton CA, Evans GJ, Sweeney ST, et al. Dopaminergic expression of the Parkinsonian gene LRRK2-G2019S leads to non-autonomous visual neurodegeneration, accelerated by increased neural demands for energy. Hum Mol Genet. 2013;22(11):2129-40.
127. Grabowski GA. Phenotype, diagnosis, and treatment of Gaucher's disease. Lancet. 2008;372(9645):1263-71.
128. Fishbein I, Kuo YM, Giasson BI, Nussbaum RL. Augmentation of phenotype in a transgenic Parkinson mouse heterozygous for a Gaucher mutation. Brain. 2014;137(Pt 12):3235-47.
129. Maor G, Rencus-Lazar S, Filocamo M, Steller H, Segal D, Horowitz M. Unfolded protein response in Gaucher disease: from human to Drosophila. Orphanet J Rare Dis. 2013;8:140.
130. Davis MY, Trinh K, Thomas RE, Yu S, Germanos AA, Whitley BN, et al. Glucocerebrosidase Deficiency in Drosophila Results in alpha-Synuclein-Independent Protein Aggregation and Neurodegeneration. PLoS Genet. 2016;12(3):e1005944.
131. Kawasaki H, Suzuki T, Ito K, Takahara T, Goto-Inoue N, Setou M, et al. Minos-insertion mutant of the Drosophila GBA gene homologue showed abnormal phenotypes of climbing ability, sleep and life span with accumulation of hydroxy-glucocerebroside. Gene. 2017;614:49-55.
132. Maor G, Cabasso O, Krivoruk O, Rodriguez J, Steller H, Segal D, et al. The contribution of mutant GBA to the development of Parkinson disease in Drosophila. Hum Mol Genet. 2016;25(13):2712-27.
133. Suzuki M, Fujikake N, Takeuchi T, Kohyama-Koganeya A, Nakajima K, Hirabayashi Y, et al. Glucocerebrosidase deficiency accelerates the accumulation of proteinase K-resistant alpha-synuclein and aggravates neurodegeneration in a Drosophila model of Parkinson's disease. Hum Mol Genet. 2015;24(23):6675-86.
134. Sanchez-Martinez A, Beavan M, Gegg ME, Chau KY, Whitworth AJ, Schapira AH. Parkinson disease-linked GBA mutation effects reversed by molecular chaperones in human cell and fly models. Sci Rep. 2016;6:31380.
135. Schondorf DC, Ivanyuk D, Baden P, Sanchez-Martinez A, De Cicco S, Yu C, et al. The NAD+ Precursor Nicotinamide Riboside Rescues Mitochondrial Defects and Neuronal Loss in iPSC and Fly Models of Parkinson's Disease. Cell Rep. 2018;23(10):2976-88.
136. Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. Proc Natl Acad Sci U S A. 1975;72(5):1858-62.

137. Orr ME, Sullivan AC, Frost B. A Brief Overview of Tauopathy: Causes, Consequences, and Therapeutic Strategies. Trends Pharmacol Sci. 2017;38(7):637-48.
138. Poorkaj P, Bird TD, Wijsman E, Nemens E, Garruto RM, Anderson L, et al. Tau is a candidate gene for chromosome 17 frontotemporal dementia. Ann Neurol. 1998;43(6):815-25.
139. van Swieten J, Spillantini MG. Hereditary frontotemporal dementia caused by Tau gene mutations. Brain Pathol. 2007;17(1):63-73.
140. Mackenzie IR, Neumann M, Bigio EH, Cairns NJ, Alafuzoff I, Kril J, et al. Nomenclature and nosology for neuropathologic subtypes of frontotemporal lobar degeneration: an update. Acta Neuropathol. 2010;119(1):1-4.
141. Galpern WR, Lang AE. Interface between tauopathies and synucleinopathies: A tale of two proteins. Annals of Neurology. 2006;59(3):449-58.
142. Rhodes SL, Sinsheimer JS, Bordelon Y, Bronstein JM, Ritz B. Replication of GWAS associations for GAK and MAPT in Parkinson's disease. Ann Hum Genet. 2011;75(2):195-200.
143. Moussaud S, Jones DR, Moussaud-Lamodiere EL, Delenclos M, Ross OA, McLean PJ. Alpha-synuclein and tau: teammates in neurodegeneration? Mol Neurodegener. 2014;9:43.
144. Baker M, Litvan I, Houlden H, Adamson J, Dickson D, Perez-Tur J, et al. Association of an extended haplotype in the tau gene with progressive supranuclear palsy. Hum Mol Genet. 1999;8(4):711-5.
145. Pastor P, Ezquerra M, Tolosa E, Munoz E, Marti MJ, Valldeoriola F, et al. Further extension of the H1 haplotype associated with progressive supranuclear palsy. Mov Disord. 2002;17(3):550-6.
146. Stefansson H, Helgason A, Thorleifsson G, Steinthorsdottir V, Masson G, Barnard J, et al. A common inversion under selection in Europeans. Nat Genet. 2005;37(2):129-37.
147. Martin ER, Scott WK, Nance MA, Watts RL, Hubble JP, Koller WC, et al. Association of single-nucleotide polymorphisms of the tau gene with late-onset Parkinson disease. JAMA. 2001;286(18):2245-50.
148. Pastor P, Ezquerra M, Munoz E, Marti MJ, Blesa R, Tolosa E, et al. Significant association between the tau gene A0/A0 genotype and Parkinson's disease. Ann Neurol. 2000;47(2):242-5.
149. Pittman AM, Fung HC, de Silva R. Untangling the tau gene association with neurodegenerative disorders. Hum Mol Genet. 2006;15 Spec No 2:R188-95.
150. Kwok JB, Teber ET, Loy C, Hallupp M, Nicholson G, Mellick GD, et al. Tau haplotypes regulate transcription and are associated with Parkinson's disease. Ann Neurol. 2004;55(3):329-34.
151. Myers AJ, Pittman AM, Zhao AS, Rohrer K, Kaleem M, Marlowe L, et al. The MAPT H1c risk haplotype is associated with increased expression of tau and especially of 4 repeat containing transcripts. Neurobiol Dis. 2007;25(3):561-70.
152. Trabzuni D, Wray S, Vandrovcova J, Ramasamy A, Walker R, Smith C, et al. MAPT expression and splicing is differentially regulated by brain region: relation to genotype and implication for tauopathies. Hum Mol Genet. 2012;21(18):4094-103.
153. Gistelinck M, Lambert JC, Callaerts P, Dermaut B, Dourlen P. Drosophila models of tauopathies: what have we learned? Int J Alzheimers Dis. 2012;2012:970980.

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3343; doi:10.3390/ijms19113343

154. Papanikolopoulou K, Skoulakis EM. The power and richness of modelling tauopathies in Drosophila. Mol Neurobiol. 2011;44(1):122-33.
155. Burnouf S, Gronke S, Augustin H, Dols J, Gorsky MK, Werner J, et al. Deletion of endogenous Tau proteins is not detrimental in Drosophila. Sci Rep. 2016;6:23102.
156. Roy B, Jackson GR. Interactions between Tau and alpha-synuclein augment neurotoxicity in a Drosophila model of Parkinson's disease. Hum Mol Genet. 2014;23(11):3008-23.
157. Caffrey TM, Wade-Martins R. Functional MAPT haplotypes: bridging the gap between genotype and neuropathology. Neurobiol Dis. 2007;27(1):1-10.
158. Zabetian CP, Hutter CM, Factor SA, Nutt JG, Higgins DS, Griffith A, et al. Association analysis of MAPT H1 haplotype and subhaplotypes in Parkinson's disease. Ann Neurol. 2007;62(2):137-44.
159. Pankratz N, Beecham GW, DeStefano AL, Dawson TM, Doheny KF, Factor SA, et al. Meta-analysis of Parkinson's disease: identification of a novel locus, RIT2. Ann Neurol. 2012;71(3):370-84.
160. Tseng WE, Chen CM, Chen YC, Yi Z, Tan EK, Wu YR. Genetic variations of GAK in two Chinese Parkinson's disease populations: a case-control study. PLoS One. 2013;8(6):e67506.
161. Shimizu H, Nagamori I, Yabuta N, Nojima H. GAK, a regulator of clathrin-mediated membrane traffic, also controls centrosome integrity and chromosome congression. J Cell Sci. 2009;122(Pt 17):3145-52.
162. Dumitriu A, Pacheco CD, Wilk JB, Strathearn KE, Latourelle JC, Goldwurm S, et al. Cyclin-G-associated kinase modifies alpha-synuclein expression levels and toxicity in Parkinson's disease: results from the GenePD Study. Hum Mol Genet. 2011;20(8):1478-87.
163. Song L, He Y, Ou J, Zhao Y, Li R, Cheng J, et al. Auxilin Underlies Progressive Locomotor Deficits and Dopaminergic Neuron Loss in a Drosophila Model of Parkinson's Disease. Cell Rep. 2017;18(5):1132-43.
164. Davisson MT. Discovery genetics: serendipity in basic research. ILAR J. 2005;46(4):338-45.
165. Keller MF, Saad M, Bras J, Bettella F, Nicolaou N, Simon-Sanchez J, et al. Using genome-wide complex trait analysis to quantify 'missing heritability' in Parkinson's disease. Hum Mol Genet. 2012;21(22):4996-5009.
166. Hoshino M, Nakamura S, Mori K, Kawauchi T, Terao M, Nishimura YV, et al. Ptf1a, a bHLH transcriptional gene, defines GABAergic neuronal fates in cerebellum. Neuron. 2005;47(2):201-13.
167. Ono Y, Nakatani T, Minaki Y, Kumai M. The basic helix-loop-helix transcription factor Nato3 controls neurogenic activity in mesencephalic floor plate cells. Development. 2010;137(11):1897-906.
168. Pascual M, Abasolo I, Mingorance-Le Meur A, Martinez A, Del Rio JA, Wright CV, et al. Cerebellar GABAergic progenitors adopt an external granule cell-like phenotype in the absence of Ptf1a transcription factor expression. Proc Natl Acad Sci U S A. 2007;104(12):5193-8.
169. Abruzzi KC, Zadina A, Luo W, Wiyanto E, Rahman R, Guo F, et al. RNA-seq analysis of Drosophila clock and non-clock neurons reveals neuron-specific cycling and novel candidate neuropeptides. PLoS Genet. 2017;13(2):e1006613.
170. Kula-Eversole E, Nagoshi E, Shang Y, Rodriguez J, Allada R, Rosbash M. Surprising gene expression patterns within and between PDF-containing

circadian neurons in Drosophila. Proc Natl Acad Sci U S A. 2010;107(30):13497-502.
171. Nagoshi E, Sugino K, Kula E, Okazaki E, Tachibana T, Nelson S, et al. Dissecting differential gene expression within the circadian neuronal circuit of Drosophila. Nat Neurosci. 2010;13(1):60-8.
172. Croset V, Treiber CD, Waddell S. Cellular diversity in the Drosophila midbrain revealed by single-cell transcriptomics. Elife. 2018;7.
173. Tas D, Stickley L, Miozzo F, Koch R, Loncle N, Sabado V, et al. Parallel roles of transcription factors dFOXO and FER2 in the development and maintenance of dopaminergic neurons. PLoS Genet. 2018;14(3):e1007271.
174. Eijkelenboom A, Burgering BM. FOXOs: signalling integrators for homeostasis maintenance. Nat Rev Mol Cell Biol. 2013;14(2):83-97.
175. Dumitriu A, Latourelle JC, Hadzi TC, Pankratz N, Garza D, Miller JP, et al. Gene expression profiles in Parkinson disease prefrontal cortex implicate FOXO1 and genes under its transcriptional regulation. PLoS Genet. 2012;8(6):e1002794.
176. Domanskyi A, Alter H, Vogt MA, Gass P, Vinnikov IA. Transcription factors Foxa1 and Foxa2 are required for adult dopamine neurons maintenance. Front Cell Neurosci. 2014;8:275.
177. Hwang S, Song S, Hong YK, Choi G, Suh YS, Han SY, et al. Drosophila DJ-1 decreases neural sensitivity to stress by negatively regulating Daxx-like protein through dFOXO. PLoS Genet. 2013;9(4):e1003412.
178. Kanao T, Venderova K, Park DS, Unterman T, Lu B, Imai Y. Activation of FoxO by LRRK2 induces expression of proapoptotic proteins and alters survival of postmitotic dopaminergic neuron in Drosophila. Hum Mol Genet. 2010;19(19):3747-58.
179. Kittappa R, Chang WW, Awatramani RB, McKay RD. The foxa2 gene controls the birth and spontaneous degeneration of dopamine neurons in old age. PLoS Biol. 2007;5(12):e325.
180. Koh H, Kim H, Kim MJ, Park J, Lee HJ, Chung J. Silent information regulator 2 (Sir2) and Forkhead box O (FOXO) complement mitochondrial dysfunction and dopaminergic neuron loss in Drosophila PTEN-induced kinase 1 (PINK1) null mutant. J Biol Chem. 2012;287(16):12750-8.
181. Pino E, Amamoto R, Zheng L, Cacquevel M, Sarria JC, Knott GW, et al. FOXO3 determines the accumulation of alpha-synuclein and controls the fate of dopaminergic neurons in the substantia nigra. Hum Mol Genet. 2014;23(6):1435-52.
182. Pristera A, Lin W, Kaufmann AK, Brimblecombe KR, Threlfell S, Dodson PD, et al. Transcription factors FOXA1 and FOXA2 maintain dopaminergic neuronal properties and control feeding behavior in adult mice. Proc Natl Acad Sci U S A. 2015;112(35):E4929-38.
183. Hartenstein V, Cruz L, Lovick JK, Guo M. Developmental analysis of the dopamine-containing neurons of the Drosophila brain. J Comp Neurol. 2017;525(2):363-79.