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**Abstract**

To date, a truly representative animal model of Parkinson disease (PD) remains a critical unmet need. Although toxin-induced PD models have served many useful purposes, they have generally failed to recapitulate accurately the progressive process as well as the nature and distribution of the human pathology. During the last decade or so, the identification of several genes whose mutations are causative of rare familial forms of PD has heralded in a new dawn for PD modelling. Numerous mammalian as well as non mammalian models of genetically-linked PD have since been created. However, despite initial optimism, none of these models turned out to be a perfect replica of PD. Meanwhile, genetic and toxin-induced models alike continue to evolve towards mimicking the disease more faithfully. Notwithstanding this, current genetic models have collectively illuminated several important pathways relevant to PD pathogenesis. Here, we have attempted to provide a comprehensive discussion on existing genetic models of PD.

## Introduction

Parkinson disease (PD) is a prevalent neurodegenerative disorder affecting millions of elderly individuals worldwide. Clinically, patients with PD exhibit a characteristic motor phenotype commonly referred to as “parkinsonism” that includes bradykinesia, postural instability, rigidity and tremor [1]. Although PD is the major cause of parkinsonism, it is important to note that the disease is clinically and pathologically distinguishable from other forms of parkinsonism. The principal neuropathology that gives rise to the constellation of primary motoric deficits seen in PD patients is the loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc) of the midbrain. This lesion results in a severe depletion of striatal dopamine (DA) and thereby an impaired nigro-striatal system that otherwise allows an individual to execute properly coordinated movements. Accordingly, pharmacological replacement of brain DA represents an effective symptomatic recourse for the PD patient, at least during the initial stages of the disease. Thus, on a practical note, one might regard the SN pathology as the most important hallmark of PD. However, it is imperative for us to recognize that neurodegeneration in PD frequently involves other areas of the brain such as the dorsal motor nucleus of the vagus, locus ceruleus (LC) and olfactory nuclei [2, 3], and that non-motor features arising from these extra-nigral neuronal losses can be additional sources of considerable consternation and disability for affected individuals [1]. Given this, one should perhaps consider PD as a multi-system disorder. Another key histological feature of PD is the presence of Lewy bodies (LBs) in surviving neurons [4]. Despite decades of research, little is known to date about how LBs are formed or whether these inclusion bodies represent a cause, consequence or an epiphenomenon of the disease.

Notwithstanding this, Braak et al have recently used LB as a marker for PD pathogenesis and enumerated six neuropathological stages of the disease which in essence, supports the notion that PD is a multi-system disorder [2]. However, it should be noted that a correlation between neuronal loss and LB burden is not taken into account in the Braak et al's paper, and that emerging evidence suggests that LB biogenesis may be a neuroprotective response [5, 6].

Given its highly complex nature, it is apparently a tremendous challenge to model PD accurately in experimental animals. Furthermore, the etiology of sporadic PD is less than straightforward, potentially involving combinations of the aging process, genetic propensity and environmental exposures [7-10]. Notably, before the advent of genetic PD models, the majority of studies have relied on neurotoxin intoxication to model PD-associated neuronal loss in animals. These neurotoxin-based models have generally failed to recapitulate accurately the progressive process that involves multiple areas of the central nervous system dominated by the hallmark aggregation of proteins into LBs in remaining neurons [11]. Another common critique of these models is the acute nature of the neurotoxin treatment paradigm, which often "hurry" the degenerative process that normally requires decades to surface in humans to mere days or weeks in an animal's lifetime. Although such approaches are useful for pragmatic reasons, the caveat is that the mechanism underlying acute neurotoxicity may not be reflective of that occurring in PD, where a slower pace of neuronal death is seen. Clearly, a chronic disease model would be more desirable. Indeed, toxin-induced models of PD seem to be evolving towards this direction. Notably, a recent study by Fornai et al demonstrated that continuous low-level

exposure of mice to the parkinsonian neurotoxin, MPTP, mimics many features of the disease including progressive behavioural changes and formation of nigral inclusion [12].

During the last decade or so, the identification of several genes whose mutations are causative of rare familial forms of PD has heralded in a new dawn for PD modelling. Genetic models, unlike their toxin-based counterparts, hold promise of reproducing the chronic nature of the degenerative process as well as the nature and distribution of the human pathology. However, despite initial optimism, the endeavour insofar to generate a representative model of PD based on the manipulation of PD-linked genes in experimental animals has proven to be challenging. Nonetheless, a wealth of useful information regarding the molecular events that surround PD pathogenesis is created along with every new PD-linked gene being modelled. Here, we have attempted to provide a comprehensive review on existing genetic models of PD and discuss the valuable insights obtained from these models.

### **Genetic models of PD**

The first gene to be unequivocally linked to familial PD is *α-synuclein* [13]. Following this discovery in 1997, several other familial PD-linked genes associated with autosomal dominant or recessive forms of PD were subsequently identified. These include *parkin* [14], *DJ-1* [15], *PINK1* [16], and *LRRK2* [17, 18]. It is noteworthy to mention here that although mutations in *UCHL1* [19] and *Omi/HtrA2* [20] have also been suggested to cause parkinsonism, their relevance to PD is currently contentious because the purported disease-causing mutation is either extremely rare in occurrence (e.g. UCHL1 I93M) or present in control population at similar frequencies (e.g. Omi/HtrA2

G399S) [21]. As for the recently-described *ATP13A2*-linked parkinsonism [22], the associated clinical phenotype (characterized by mild parkinsonism and prominent cognitive defects) is rather divergent from classical PD and its relevance to PD is similarly debatable. In general, PD-linked genes are expressed as transgenes in heterologous organisms if their inheritance pattern in humans suggests a dominant transmission. Otherwise, orthologous copies of the human gene are ablated in animals to mimic recessive loss of gene function. To date, several mouse models of familial parkinsonism, as well as fly and worm models, have been generated. Although the non-mammalian PD models cannot recapitulate fully the phenotypic and pathologic features of the human condition, they are nonetheless able to reproduce certain salient hallmarks of PD such as LB-like inclusions and DA neurodegeneration [23, 24], and are therefore useful models to study the relationship between PD-linked genes and DA neuronal function. Notably, six DA neuronal clusters are recognizable in each of the adult fly brain hemispheres (Fig. 1A), while a total of eight DA neurons organized into three subsets are found in the *C. elegans* (Fig. 1B). Importantly, the well-characterized genetics of these non-mammalian PD models offer a unique advantage over the mouse model for the rapid identification of modifiers that could illuminate important pathways involved in disease pathogenesis, insights of which could facilitate the subsequent development of novel therapeutics.

### **Alpha-synuclein**

$\alpha$ -Synuclein is a small (140 amino acids) but abundant neuronal protein that is particularly enriched in pre-synaptic terminals. Although the precise physiological



function of  $\alpha$ -synuclein remains to be elucidated, several studies have suggested a regulatory role for the protein in membrane and vesicular dynamics [25, 26]. It is currently well established that mutations in the  $\alpha$ -synuclein gene, occurring as substitutions (A53T, A30P and E46K), duplication or triplication, are causative of dominantly inherited forms of PD [13, 27-29]. Importantly,  $\alpha$ -synuclein has been identified to be a major structural component of LBs [30]. Collectively, these findings provide a compelling case for modelling PD via the over expression of wild type or mutant forms of  $\alpha$ -synuclein in animals.

Masliah and colleagues developed the first transgenic mouse model that over expresses wild type human  $\alpha$ -synuclein [31]. They observed in these mice a progressive accumulation of neuronal inclusions in the neocortex, hippocampus and SN that stained positively with antibodies to  $\alpha$ -synuclein and ubiquitin. However, these inclusions are not of a fibrillar composition that otherwise characterizes LBs. Further, there is no apparent loss of DA neurons in these mice. Although the highest-expressing line exhibits significant degeneration of TH-positive nerve terminals within the striatum that is accompanied by motor impairment on a rotarod, the degeneration pattern is distinct from the profound DA cell loss seen in PD. Moreover, the observed phenomenon is dissociated from inclusions formation, as other neuronal subtypes in same line and also the same subset of neurons in other lines that are all burdened by inclusions are spared from degeneration. However, as mentioned earlier, it is currently unclear whether neuronal loss and inclusions load are linked in a causative manner. Notwithstanding this, the  $\alpha$ -synuclein mouse model of PD generated by Masliah and colleagues, although insightful, does not appear to recapitulate faithfully the human pathology. This less than robust PD

phenotype exhibited by Masliah's mice is essentially a recurring theme of many more  $\alpha$ -synuclein mouse models that followed, which differ principally from each other in the promoter used and whether the human  $\alpha$ -synuclein transgene is a wild type or mutated copy (Table 1). It is noteworthy that several of these models that relied on the prion or Thy-1 promoter to drive high levels of wild type or mutant  $\alpha$ -synuclein expression produce widespread extra-nigral pathology, including motor neuron degeneration and glial pathology in some, which on the whole is less reflective of PD than of synucleinopathies [32-35]. To achieve a more localized effect of  $\alpha$ -synuclein over expression, a number of groups have generated  $\alpha$ -synuclein lines using the TH promoter, which directs expression specifically in catecholaminergic neurons. Notwithstanding the obvious limitation of this approach in modelling the broad  $\alpha$ -synuclein pathology that characterizes the human disease, TH-driven wild type, A53T or A30P  $\alpha$ -synuclein mice similarly fail to enhance DA cell loss [36, 37]. Further, these mice also tend to lack  $\alpha$ -synuclein-positive inclusions [36, 37]. A notable exception is a TH-synuclein line that expresses a doubly mutated (A30P/A53T) form of human  $\alpha$ -synuclein, which represents one the few genetic models to date to show an apparent, age-dependent, loss of TH-positive neurons in the SNpc that is accompanied by a decline in motor activity, albeit in the absence of detectable neuronal aggregates [38]. However, it is currently unclear whether the observed phenomenon could have arisen from a regulatory effect of the  $\alpha$ -synuclein double mutant on TH expression. Further, the patho-physiological relevance of doubly mutated  $\alpha$ -synuclein is questionable, as none has reported its occurrence in familial PD cases thus far. Nonetheless, these mice are potentially useful for understanding how aberrant forms of  $\alpha$ -synuclein could trigger DA neurotoxicity. More

recently, two groups have independently generated transgenic mice over expressing truncated forms of  $\alpha$ -synuclein under the direction of the TH promoter [39, 40]. The motivation for this came from several previous *in vitro* studies suggesting that C-terminally truncated  $\alpha$ -synuclein ( $\alpha$ -Syn $\Delta$ C) may be pathologically relevant [41-44], as well as from the observation by Lee et al that these proteolytically-processed forms of  $\alpha$ -synuclein accumulate in A53T-synuclein-expressing mice specifically in areas of the brain undergoing active neurodegeneration [33]. Moreover, in a follow-up study, the group led by Lee demonstrated that  $\alpha$ -Syn $\Delta$ C species are enriched in  $\alpha$ -synuclein-positive aggregates and that disease-associated mutations promote their accumulation [45]. Further,  $\alpha$ -Syn $\Delta$ C enhances the *in vitro* aggregation of the full-length protein, thereby promoting its dysfunction. Interestingly, one of the truncated  $\alpha$ -synuclein models generated that expresses a 130 amino acid-containing version of the protein exhibit a remarkable and selective degeneration of nigral DA neurons that is associated with impaired locomotory function [40]. Importantly, the motoric abnormality could be reversed by L-DOPA treatment, a phenomenon that recapitulates the human condition. Unfortunately, the neuronal loss is not progressive and apparently occurs during embryogenesis [40]. Moreover,  $\alpha$ -synuclein-positive inclusions are absent in these mice. Although fibrillar  $\alpha$ -synuclein inclusions are detected in the other truncated  $\alpha$ -synuclein model that expresses a 120 amino acid-containing protein, their presence is not accompanied by the loss of nigral DA neurons [39]. Meanwhile, the  $\alpha$ -synuclein mouse model continues to evolve. Earlier this year, Nuber and colleagues described a novel transgenic  $\alpha$ -synuclein mouse model that expresses the wild type protein under a tetracycline (tet)-regulatable system [46]. The Germany-based investigators demonstrated

in these mice a modest loss of neurons principally in the reticulata zone of the SN that is nonetheless accompanied by progressive motor decline. Further, they also observed hippocampal pathology, associated cognitive impairment, and interestingly, reduced neurogenesis in these mice upon turning on the transgene expression for a period of time. All these phenotypic alterations however occur in the absence of fibrillary inclusions. Importantly, the cessation of transgene expression mitigates progression of the phenotype but did not reverse the symptoms, suggesting that the continual expression of  $\alpha$ -synuclein is required for disease progression. It is noteworthy to mention that prior to this demonstration by Nuber et al, the question on whether disease progression mediated by the expressed transgene could be halted or reversed after its onset by turning off the transgene expression could not be explored. The conditional model that Nuber and colleagues have generated thus represents an important advance in  $\alpha$ -synuclein modelling.

In summary, despite the initial excitement, none of the  $\alpha$ -synuclein mouse models generated thus far replicates the human disease faithfully. Indeed, fibrillary inclusions are found lacking in the majority of these models, and SNpc cell loss is, more often than not, non-existent in  $\alpha$ -synuclein-expressing mice. Some investigators have suggested that the absence of frank nigral pathology in the various  $\alpha$ -synuclein mouse models is due to the inherent resistance of rodent's nigral neurons to degeneration [33]. This is debatable, as the expression of both the truncated and doubly-mutated forms of  $\alpha$ -synuclein could promote the loss of TH-positive neurons in the SNpc [38, 40]. Moreover, direct delivery of  $\alpha$ -synuclein-containing viral vectors into the SN of rats and mice alike via stereotactic injections result in DA neurodegeneration [47-49]. Indeed, the use of viral vectors to

over-express PD-linked genes represents an alternative and important way to model PD in rodents (Excellent reviews on this topic have been written elsewhere, for example see [50]). At least in rodents, it thus appears that a threshold expression level (achieved by viral delivery) or some modifications (achieved by truncation or double mutation) is needed for  $\alpha$ -synuclein to manifest its toxicity in DA neurons.

In sharp contrast to the general lack of PD phenotype in  $\alpha$ -synuclein mouse models, transgenic flies over expressing  $\alpha$ -synuclein surprisingly exhibit robust signs of parkinsonism. Concurrent to Masliah et al's report of the first  $\alpha$ -synuclein-expressing mice, Feany and Bender reported the first *Drosophila* model of PD that was generated via directed expression of wild type or mutant forms (A53T and A30P) of human  $\alpha$ -synuclein in the nervous system of the fly [51]. Remarkably, transgenic flies over expressing wild type or mutant  $\alpha$ -synuclein exhibit an adult-onset, age-dependent loss of dorsomedial clusters (DMCs) of DA neurons that is accompanied by a progressive loss of climbing ability. Further, these flies also developed intraneuronal  $\alpha$ -synuclein-positive inclusions that resemble LBs. Importantly, inclusions formation parallels  $\alpha$ -synuclein-induced toxicity both spatially and temporally. The fly model developed by Feany and Bender thus recapitulates the salient features of PD, including disease onset at adulthood, progressive locomotion deficit that is associated with an age-dependent and selective loss of DA neurons, and formation of fibrillar  $\alpha$ -synuclein inclusions. Given this, and its amenability to genetic screen for modifiers, the  $\alpha$ -synuclein fly model offers a valuable tool to elucidate suppressors of  $\alpha$ -synuclein-mediated toxicity. At the same time, the model also allows for the direct assessment of candidate suppressor function *in vivo*, which is less time consuming than unbiased genetic screens. Using the candidate

approach, Auluck et al demonstrated that co-expression of Hsp70 with  $\alpha$ -synuclein reduces  $\alpha$ -synuclein aggregation and concomitantly attenuates  $\alpha$ -synuclein-mediated DA neuronal death in flies, a finding corroborated later by others in mice [52, 53]. Consistent with this, transgenic flies fed with geldanamycin, an enhancer of Hsp70 expression and a modulator of stress response, similarly suppresses  $\alpha$ -synuclein toxicity [54, 55]. Together, these studies suggest a neuroprotective role of Hsp70 against  $\alpha$ -synuclein-mediated toxicity, and provide a good illustration of the utility of the *Drosophila* PD model in facilitating the discovery of compounds of therapeutic potential. Interestingly, the neuroprotective effect of geldanamycin appears to be uncoupled from inclusion formation, i.e. in the presence of geldanamycin, DA neurons are spared from degeneration despite the continued presence of inclusion pathology [55]. Consistent with this, Chen and Feany observed a reciprocal relationship between aggregate formation and neurotoxicity in flies harbouring a missense mutation (S129A) on  $\alpha$ -synuclein that renders the expressed protein inert to phosphorylation at Ser129 [56], the modification of which has been associated with fibril formation and enhanced neurotoxicity [57]. Notably, the S129A mutation markedly increases inclusion body formation and also abrogates  $\alpha$ -synuclein toxicity. Conversely, the alteration of Ser129 to the negatively charged residue aspartate that mimics phosphorylation apparently favors the maintenance of  $\alpha$ -synuclein in a soluble, toxic form and significantly enhances  $\alpha$ -synuclein-mediated toxicity in flies. Thus, the phosphorylation status of Ser129 appears crucial in mediating  $\alpha$ -synuclein neurotoxicity and inclusion formation. However, in mice expressing full length  $\alpha$ -synuclein, this residue appears to be constitutively phosphorylated but the

modification alone is clearly insufficient to trigger neurodegeneration in these animals [58].

As with the mouse models, controversies also surround the *Drosophila*  $\alpha$ -synuclein model. Whereas Feany and Bender reported complete (or near complete) loss of DA neurons in the DMCs in 30-day-old transgenic flies [51], Auluck et al observed only a 50% loss in the same subset of DA neurons [52], a finding later corroborated by another group [59]. Notably, paraffin sections of fly brains were analyzed in these studies. A more recent study that assayed DA neurons in  $\alpha$ -synuclein-expressing flies by means of whole-mount immunohistochemistry coupled with confocal microscopy (which allowed the simultaneous viewing of all DMC neurons in a single preparation) failed to detect any significant DA cell loss [60]. Given these discrepancies, one needs to interpret the results obtained from various *Drosophila*  $\alpha$ -synuclein models with caution, particularly when the choice of assay could potentially influence the observation made. In an attempt to optimize the method for the histological detection of DA neurons in flies, Drobysheva et al has very recently demonstrated that discrepancies in DA neuronal staining could arise from the fixative used, method of fixative delivery, sectioning technique and choice of antibodies [61]. Notably, paraffin sections were found to be inconsistent in preserving DA epitopes. In the same report, the authors also suggested an optimized protocol for DA neuronal staining in *Drosophila* that combines a weak fixative with cooled microwave infiltration, buffers that provide anti-oxidant protection of DA epitopes and agarose embedding coupled with thick sectioning on a vibratome. Whether the described method for DA neurons detection in flies is more sensitive than confocal microscopy-aided whole mount analysis currently awaits evaluation by other groups.

Along with fly and mouse models, PD investigators have also created *C. elegans* expressing  $\alpha$ -synuclein. Lakso and colleagues reported a *C. elegans*  $\alpha$ -synuclein model that over expresses wild type or mutant (A53T) forms of the protein [24]. They demonstrated in these transgenic worms an accelerated loss of all three subsets of the nematode's DA neurons following DA neuronal-specific expression of  $\alpha$ -synuclein. However, this genetic ablation of DA neurons is neither accompanied by inclusions formation nor motoric deficits. Notably, DA neurons in *C. elegans* are thought to function as mechanosensors that have the capacity to modulate but not control movements. Consistent with this, pan-neuronal or motor neuronal-specific expression of  $\alpha$ -synuclein in *C. elegans* results in significant motor neuron degeneration and concomitantly compromised its motor ability [24]. A subsequent model developed by Kuwahara et al which over-expresses mutant A53T or A30P human  $\alpha$ -synuclein in a DA neuron-specific manner exhibits abnormal food sensing behaviour linked to DA neuronal dysfunction, albeit without overt cell loss [62]. This behavioural abnormality was accompanied by a reduction in neuronal DA content and was alleviated by the administration of exogenous DA. Similar to Lakso's model, the investigators did not detect the presence of LB-like inclusions in these worms. More recently, Ham et al described a *C. elegans*  $\alpha$ -synuclein model that developed inclusions, but in its body wall muscles because a muscle-specific promoter was used to drive the transgene expression [63]. Importantly, a genome wide RNAi screening performed revealed suppressor roles of several age-related genes, including sir-2.1/SIRT1 and lagr-1/LASS2, in  $\alpha$ -synuclein-mediated inclusions formation, thereby suggesting a link between cellular aging and inclusions biogenesis. In a separate RNAi screen and as a follow-up study, Kuwahara et



al uncovered four genes related to the endocytic pathways that cause severe growth/motor abnormalities selectively in  $\alpha$ -synuclein transgenic worms [64]. This finding suggests a relationship between aberrant endocytosis and  $\alpha$ -synuclein-mediated neuronal dysfunction. Together, the worm and fly models of  $\alpha$ -synuclein provide further support for a neurotoxic role of  $\alpha$ -synuclein accumulation, and importantly, helped to illuminate potential pathways involved in  $\alpha$ -synuclein-mediated pathology.

### **Parkin**

Shortly after the discovery of  $\alpha$ -synuclein as a familial PD-linked gene, mutations in the parkin gene were reported to be causative of autosomal recessive juvenile parkinsonism (ARJP) [14]. Following this association, three independent groups demonstrated that parkin functions as a ubiquitin ligase associated with proteasomal degradation and that disease-causing mutations of parkin compromise its normal role as an E3 enzyme [65-67]. A logical working hypothesis that ensued is that loss of parkin function leads to a neurotoxic accumulation of one or several of its substrates. Indeed, numerous reported substrates of parkin, including CDCrel1 [68], CDCrel2a [68], Cyclin E [69], Pael-R [70], AIMP2/p38 [71, 72] and FBP1 [73], were later found to accumulate in the brains of PD patients carrying parkin mutations. However, as none of the parkin substrates identified thus far is exclusively expressed in DA neurons, it remains puzzling why DA neurons in familial parkinsonism linked to parkin mutations are selectively vulnerable to deficient parkin function. Nonetheless, because parkin mutations are classically transmitted in a recessive manner, there was much optimism amongst PD investigators to create a representative model mimicking parkin-related parkinsonism

through the ablation of orthologous parkin gene in the animal. Further, because parkin-related cases are usually devoid of classic LBs [74-76], assessment of the anticipated pathology in parkin null mice also appears more straightforward.

In 2003, Goldberg et al and Itier et al independently reported the characterization of parkin null mice that were created via the deletion of murine parkin exon 3 [77, 78]. Soon after, two other groups respectively generated exon 2- and exon-7-deleted parkin mice [79, 80]. Although all of them confirmed the abolition of parkin mRNA and protein expression in their respective models, none of these parkin null models exhibit apparent signs of parkinsonism (Table 2). Consistent with this, the neurological mouse mutant *quaking<sup>viable</sup>* (*qk<sup>v</sup>*), which was recently identified to be parkin-deficient due to the spontaneous deletion of a genetic region that consisted of the murine parkin gene, displays no loss of DA neurons [81, 82]. Furthermore, despite the association of parkin function with protein degradation, the majority of previously identified parkin substrates (except AIMP2/p38 and FBP1) do not show any detectable accumulation in these mice [72, 73, 77-80]. Although the levels of AIMP2/p38 and FBP1 are elevated in the brains of parkin null mice [72, 73], this phenomenon is currently confined to the parkin exon 7-deleted line, i.e. whether the same is observed in other parkin knockout lines awaits confirmation. Interestingly, the same line also exhibits noradrenergic dysfunction due to a significant, albeit non-progressive, loss of locus coeruleus (LC) neurons [80]. However, it is not entirely clear at this moment why this deficit is not seen in other lines. Moreover, parkin-deficient *qk<sup>v</sup>* mutant exhibit an opposite phenotype, i.e. increase in LC neurons [83]. In the case of exon 3-deleted parkin mice, biochemical and proteomic analyses of brain samples from these mice revealed deficits in dopamine handling, reduced

mitochondrial respiration and antioxidant capacity, as well as increased ROS-mediated damage [77, 78, 84]. Further, primary midbrain neuronal cultures prepared from one of these lines exhibit increased susceptibility to neurotoxicity elicited by rotenone, a PD-linked mitochondrial poison, compared to control cultures [85]. These features are consistent with the implicated cellular roles of parkin in regulating mitochondrial function and conferring protection against oxidative stress [86-89]. Further supporting this, virus-mediated delivery of parkin prevents dopaminergic neurodegeneration in rats either overexpressing  $\alpha$ -synuclein [90, 91] or treated with 6-OHDA [92], as well as in mice treated with MPTP [93], although conflicting observations have also been reported [94]. In the *Drosophila* system, we and others have similarly observed an intimate functional relationship between parkin and the mitochondria (discussed below). Indeed, mitochondrial pathology seems to be the central characteristic of a variety of *Drosophila* parkin mutants generated thus far.

The *Drosophila* parkin gene encodes a protein that bears a high degree of sequence similarity to the human parkin protein [95, 96]. Overall, the amino acid identity between fly and human parkin is 46%, but the sequence conservation between their respective catalytic (RING-IBR-RING) region is even higher (56% amino acid identity), suggesting an evolutionary conserved function. Using an established P-element mutagenesis strategy, the group led by Leo Pallanck originally developed flies devoid of endogenous parkin gene [95]. These *parkin* null flies are semi-viable and display reduced lifespan, locomotor defects and male sterility. However, the initial assessment of TH-positive neurons in paraffin-embedded head sections revealed no obvious loss of DA neurons, although cells in the DMCs showed shrinkage of the cell body and decreased

TH-staining [97]. These observations were subsequently corroborated by another group, who further found that c-Jun terminal kinase (JNK) is activated in the dorsomedial DA neurons of parkin null flies, thereby suggesting a negative role for parkin in JNK signalling pathway and at the same time, a pathogenic mechanism involving JNK activation in parkin-related neurodegeneration [97]. The Pallanck's group later discovered that a substantial variance in sample analysis might have obscured a subtle loss of neurons in their parkin null fly model. Following a re-analysis by the same group using the more sensitive, confocal microscopy-based quantification method, the investigators demonstrated in parkin null flies a significant and progressive degeneration of a subset of DA neurons known as the protocerebral posterior lateral (PPL1) cluster [98]. However, a question that remained was whether the loss of select subsets of DA neurons is sufficient to trigger locomotion defects in the *Drosophila*, as parkin null flies also exhibit marked defects in their musculature, which is plagued by pronounced mitochondrial pathology and muscle degeneration [95, 96]. Nonetheless, mitochondrial dysfunction is commonly associated with sporadic PD pathogenesis and it is conceivable that the mechanism underlying muscle degeneration in flies may be relevant to DA neurodegeneration. Moreover, as discussed above, aberrant mitochondrial function also appears to be a feature of parkin null mice. Further, in a related study, the deletion of parkin exons 1-4 in *C. elegans* produces a mutant that show selective vulnerability to mitochondrial complex I inhibitors [99], again implicating a functional relationship between parkin and the mitochondrion. Thus, the disruption of mitochondrial function appears to be a common feature of parkin null animals. To elucidate the mechanism responsible for the muscle pathology in parkin null flies, the Pallanck's group performed

a genetic screen for parkin modifiers and found that loss-of-function mutations in oxidative stress response components enhance the phenotype of parkin mutant flies [100]. One of these components is glutathione S-transferase S1 (GstS1), an important cellular defence component against oxidative stress. In a separate study by the same group, the investigators found that overexpression of GstS1 also prevents DA neurodegeneration in parkin mutant flies, suggesting that the induction of GstS1 expression could be useful in the treatment of parkin-related PD [98].

Although the ablation of orthologous parkin gene in animals is an appropriate approach to model its apparent loss-of-function in parkin-related PD, numerous reports suggest that single parkin mutations alone may confer increased susceptibility to developing the disease [101-104]. These include two case-control studies which revealed the presence of several heterozygous parkin mutations that are not found in control individuals [101, 104]. However, the genetic basis of this remains unclear, as most of the single parkin mutant allele do not appear to transmit in an autosomal dominant fashion as might be expected [105]. Two parkin mutations that frequently occur in heterozygous state are R275W and G328E. To better understand the effects of parkin mutations *in vivo*, we and others have recently generated transgenic *Drosophila* expressing various parkin mutants under the control of a DA neuron-specific driver [106, 107]. We demonstrated in R275W, but not G328E, mutant parkin-expressing flies an age-dependent loss of DA neurons from the PPL1, and also PAM, DA neuronal clusters. Importantly, by directing the expression of the parkin mutant specifically in TH-positive neurons, we found that DA neurodegeneration alone is sufficient to trigger progressive locomotion defects in these R275W parkin-expressing flies [106]. Similar observations were made by Sang et

al with transgenic flies over expressing two other disease-associated parkin mutants [107]. Our results thus suggest a direct association between DA neurodegeneration and locomotor dysfunction in flies, a phenomenon that is seen in human PD patients. It is also noteworthy to mention that when the R275W mutant is expressed specifically in the indirect flight muscles of flies, it generated pleiomorphic mitochondrial abnormalities [108]. Together, our results also suggest the interesting possibility that certain parkin mutations may directly exert neurotoxicity *in vivo*. Mechanistically, how select parkin mutants could mediate pathogenic outcomes in the *Drosophila* remains to be elucidated. However, it is tempting to propose that R275W parkin-mediated toxicity may be related to the protein's propensity to aggregate, as we and others have previously demonstrated in cultured cells [109-113]. Interestingly, *C. elegans* carrying an in-frame deletion variant of *pdr-1*, a human parkin ortholog, which exhibit altered solubility and intracellular localization properties are hypersensitive to a variety of proteotoxic conditions, including ER stress and A53T  $\alpha$ -synuclein-mediated aggregation [114]. In contrast, those that have lost their *pdr-1* expression altogether are viable and do not display obvious defects in their DA neurons [114]. These studies suggest that misfolded parkin may be a relevant pathogenic factor in at least some of the parkin-related PD cases.

## **PINK1**

Like parkin, mutations in *PINK1* are associated with recessive parkinsonism [15, 16]. However, very little is known to date about the pathology of PINK1-related cases, although the loss or defective function of DA neurons is likely to be the underlying pathology as PINK1-related patients generally respond well to L-DOPA treatment.

Several recent studies involving *pink1*-deficient flies have provided unequivocal evidence supporting a link between PINK1 mutations and mitochondrial dysfunction [115-117]. Two concurrent papers in 2006 by Clark et al and Park et al reported that *pink1*-deleted mutant *Drosophila* are characterized by dramatic mitochondrial defects in muscles and testes that include swelling and fragmentation of the organelle, as well as reduction in ATP levels and mitochondrial DNA content [115, 116]. Otherwise, these *pink1*-deficient flies are viable, although the males are sterile, and exhibit either no or mild loss of DA neurons. Importantly, because the various phenotypes of *pink1*-deficient flies bear striking resemblance to the observed characteristics of parkin mutant flies, the investigators examined whether the two PD-related genes interact with each other functionally and found that parkin over expression in *pink1*<sup>-/-</sup> flies significantly ameliorated all the mutant phenotypes tested [115, 116]. The reverse, however, does not happen, i.e. PINK1 over expression in parkin null flies does not compensate for the loss of parkin function [115, 116]. These observations were essentially corroborated by a separate but related study conducted by Yang et al that involved RNAi knockdown of *pink* in *Drosophila* [118]. Yang et al also extended the parkin rescue experiment by demonstrating that DJ-1 over expression has no mitigating effects on *pink1* mutant phenotypes. Further, the investigators showed that parkin protein level is reduced in *pink1*<sup>-/-</sup> flies. More recently, the same group also demonstrated a role for PINK1 as a regulator of mitochondrial dynamics via the protein interaction with the mitochondrial fission/fusion machinery [119]. The investigators found that whereas PINK1 over expression promotes mitochondrial fission, its inhibition results in excessive fusion of mitochondria [119]. Similar findings were reported by Poole et al at around the same

time [120]. Together, these studies provide compelling evidence supporting a role of PINK1 in maintaining mitochondrial homeostasis and function, as well as a role for parkin as an important downstream regulator of PINK1. It tempting to suggest that the interaction between parkin and PINK1 occurs not only functionally, but also physically, and in a linear pathway. Although the parkin protein resides primarily outside the mitochondrion - a purported residence of PINK1, some reports have suggested that a small amount of parkin associates with this organelle [86, 88]. Further, a recent study suggests that whilst the PINK1 protein spans the outer mitochondrial membrane, its kinase domain faces the cytoplasm [121]. Thus, a direct interaction between parkin and PINK1 (at least at the cytosolic face of the mitochondria) is not entirely inconceivable. Notably, an interaction between parkin and PINK1 has been demonstrated in a co-transfection system [122]. However, whether this interaction takes place endogenously and at the mitochondrial surface remains to be established.

Subsequent to the characterization of *Drosophila pink1* mutants, Kitada and colleagues reported the first analysis of PINK1 deficiency in mice [123] (Table 2). Similar to parkin null mice, *PINK1* knockout mice do not show any gross abnormality in the morphology and density of DA cell bodies. There is also no reported presence of inclusion pathology or locomotion defects. However, subtle nigrostriatal deficits are detectable in these mice. PINK1-deficient mice show a reduction in evoked DA release (as recorded using striatal slices) that does not appear to be associated with an increased reuptake of DA by DAT, but rather reflects an impaired release mechanism. Further, intracellular measurements of striatal medium spiny neurons revealed impairments of corticostriatal long term potentiation and long term depression that could be restored with



DA receptor agonists, L-DOPA or amphetamine, all of which promote DA neurotransmission. Since mitochondrial abnormality is the central characteristic of *pink1* null flies, Kitada et al also examined whether this feature is present in PINK1-deficient mice but had mentioned in their report the lack of overt mitochondrial morphological defects in these mice [123]. However, a follow-up study by the same group of investigators revealed an increase in the number of larger mitochondria in the striatum of PINK1 null mice [124], which supports the suggested role of PINK1 in promoting mitochondrial fission [119, 120]. Further, impaired mitochondrial respiration attributable to defects in complex I and also II-IV was also observed in the striatum of these mice. In contrast, mitochondrial respiration is not altered in the cerebral cortex of young (3-4 months) PINK1-deficient mice but is significantly decreased in older (22-24 months) knockout mice or when the young mice are treated with exogenous stressors, suggesting that aging or stress promotes mitochondrial dysfunction in these mice. Supporting this, intra-nigral virus-mediated delivery of wild type PINK1, but not its mutants, affords considerable protection against SN degeneration in mice treated with MPTP [125]. In a related development, J. Downward's group recently demonstrated that the mitochondrial serine protease, Omi/HtrA2, is phosphorylated in a PINK1-dependent manner upon activation of the p38 stress pathway [126]. As mentioned earlier, Omi/HtrA2 sequence variations have been associated with an increased risk for PD [20, 127], although a separate study revealed that the Omi G399S disease variant is also found in neurologically normal controls [21]. Interestingly, PINK1 also appears to phosphorylate the mitochondrial chaperone TRAP1 [128] as well as interacts with Hsp90 [129]. Since Omi/HtrA2, TRAP and Hsp90 all have chaperone functions, it is conceivable that the

susceptibility of PINK1-deficient mitochondria towards stress-induced impairment may be contributed by the loss of PINK1 interaction with these effectors.

### **DJ-1**

Mutations of DJ-1 associated with familial parkinsonism generally occur in a recessive fashion [15]. Whereas PINK1 has been identified to be a mitochondrial-related protein kinase [16], the exact function of DJ-1 is less clear. Several studies have suggested that DJ-1 operates as an anti-oxidant protein [130, 131]. Such a role of DJ-1 would be useful in counteracting the well-recognized oxidative environment of DA neurons. However, ablation of *DJ-1* in mice did not accelerate DA degeneration in the SN, even when the animals were aged up to 28 months [132-136] (Table 2). Further, there is neither accumulation of inclusion bodies nor loss of LC neurons in the brains of these mice [133, 134]. Instead, DJ-1 null mice exhibit significantly reduced DA release in the striatum which occurs as a result of increased reuptake of DA by the DA transporter (DAT) [132, 134, 136], although this phenomenon is not observed in all the reported DJ-1 knockout lines [135]. Consistent with this, DJ-1 null mice display generalized hypokinesia in open field tests [132, 134, 135]. Together, these observations suggest that DJ-1 deficiency could trigger motoric deficits in the absence of frank nigral neurodegeneration. However, in the absence of robust pathology data from DJ-1 patients, it is difficult to evaluate at this moment whether the phenotype of DJ-1 null mice mirrors the human condition. More recently, a related study revealed no behavioural, neurochemical or anatomical deficits in the DA system of DJ-1 null mice [137]. The same study identified DJ-1 as an atypical peroxidase that functions to

scavenge mitochondrial H<sub>2</sub>O<sub>2</sub> through oxidation of its cysteine at position 106, a residue on DJ-1 that was previously demonstrated by others via mutagenesis and structural analyses to be modified by oxidative stress [131]. Notably, a pool of DJ-1 is known to be localized to the mitochondria [131, 138]. Supporting a role for DJ-1 in the mitochondria, the investigators found increased levels of H<sub>2</sub>O<sub>2</sub> in mitochondria isolated from their DJ-1 knockout model [137]. Thus, the absence of DJ-1 may predispose DA neurons to stress-induced degeneration. Indeed, DJ-1-deficient mice and flies alike are hypersensitive to pharmacological inducers of oxidative stress [118, 136, 139-142].

Unlike the mouse, the *Drosophila* contains two orthologs of the human *DJ-1*, termed *DJ-1 $\alpha$*  and *DJ-1 $\beta$*  that are expressed differentially, i.e. whereas DJ-1 $\beta$  is ubiquitously expressed, the expression of DJ-1 $\alpha$  is restricted to the male germline [139, 140]. Flies genetically ablated of *DJ-1 $\beta$*  or both *DJ-1 $\alpha$*  and *DJ-1 $\beta$*  show a full complement of DA neurons [139, 140, 142], but the inactivation of *DJ-1 $\alpha$*  expression via RNAi resulted in an age-dependent reduction of TH-positive neurons in the DMC, as revealed by immunostaining of paraffin brain sections [118]. Although some groups have also reported locomotion deficits in DJ-1 mutant flies [142, 143], none of these studies have examined the mutant flies for mitochondrial pathology. Notwithstanding this, a consistent observation amongst the different groups is that DJ-1 mutant flies display a dramatically enhanced sensitivity to toxins that induce oxidative stress [118, 139, 140, 142], with aged flies showing further vulnerability to these agents [144]. Apparently, both the aging process and oxidation challenge promote over-oxidation of DJ-1 at Cys104 (analogous to Cys106 in human DJ-1) [144], a modification that could irreversibly inactivate the protein [137]. Collectively, these studies support a role for DJ-

1 in protecting neurons against oxidative insults, and at the same time, suggest a mechanism by which DJ-1 function could be progressively lost through aging, and thereby an increased risk for the demise of oxidation-prone DA neurons.

## **LRRK2**

LRRK2 is a large protein (2527 amino acids) comprised of several independent domains, one of which is a kinase domain capable of exhibiting a GTP-dependent phosphorylation activity [145-147]. Mutations in LRRK2 are currently recognized to be the most common genetic cause of parkinsonism, accounting for about 5-6% of patients with familial PD and an unprecedented 1-3% prevalence in sporadic PD [17, 18]. The predominantly late-onset phenotype of LRRK2 mutation-related PD is clinically largely indistinguishable from idiopathic PD, although the inclusions pathology that accompanies SN degeneration in LRRK2 patients is pleiomorphic [148]. To date, at least 20 LRRK2 mutations have been identified to be causative of autosomal dominant parkinsonism, with the G2019S missense mutation being the most prevalent [148, 149]. Several groups have demonstrated an increase kinase activity for the LRRK2 G2019S substitution, which concomitantly increases its toxicity [145-147, 150-152]. However, the phenomenon is not consistently observed for other LRRK2 mutations. Notably, some LRRK2 mutations (e.g. I2012T) appear to have reduced kinase activity instead [151], and several LRRK2 mutants also exhibit a propensity to aggregate when expressed in cultured cells [147]. Thus, the exact mechanism by which LRRK2 mutations cause disease is not entirely clear at this moment.

Because of the prevalence of LRRK2 mutations, and the similarities between LRRK2-induced parkinsonism and sporadic PD, there is much enthusiasm in modelling LRRK2 dysfunction in animals. Although a mouse model of LRRK2 has yet to be formally reported at the time of this review preparation, a number of groups have recently reported the analysis of LRRK2 mutant flies. Consistent with its demonstrated toxicity in cultured cells, Liu et al found that the expression of human LRRK2 G2019S mutant in the fly's photoreceptor cells triggers marked retinal degeneration. [153]. Interestingly, the same phenotype is observed with eye-specific expression of wild type human LRRK2, suggesting that de-regulated LRRK2 levels could also mediate pathogenic outcomes. Importantly, pan-neuronal or DA neuron-specific expression of both wild type and mutant human LRRK2 in flies results in adult-onset selective degeneration of TH-positive neurons, as measured by confocal microscopy-based analysis of whole mount fly brains. Further, these flies exhibit locomotor deficits that could be ameliorated by L-DOPA treatment. However, unlike parkin mutant flies, the observed loss of TH-positive neurons triggered by LRRK2 over expression is not restricted to select DA clusters. Instead, all the DA clusters in the fly brain are similarly affected by LRRK2 over expression. This is rather intriguing, considering that specific groups of DA neurons are affected in LRRK2-related human PD cases. In contrast, Lee et al reported that transgenic flies over expressing the fly orthologs of human wild type or disease-associated R1441C mutant LRRK2 do not exhibit any significant defects in the tissues examined, including DA neurons and muscles [154]. Instead, the ablation of endogenous dLRRK2 expression in flies result in a reduction in TH-immunostaining, specifically in the dorsomedial and posteromedial clusters [154]. However, a related study by another group revealed a

dispensable role of *Drosophila* ortholog of LRRK2 in the survival of DA neurons, although the investigators observed that *dLRRK2* *-/-* flies are selectively sensitive to H<sub>2</sub>O<sub>2</sub>-induced stress [155]. Similarly, a very recent report by Imai et al suggests that *dLRRK2* is not essential for the maintenance of DA neurons. The group also characterized transgenic flies expressing *Drosophila* orthologs of human LRRK2 Y1699C or I2020T mutants, which is of particular interest here in view of the lack of phenotype in LRRK2 mutant-expressing flies observed by Lee et al. Likewise, Imai et al reported no difference in the number of DA neurons in 10-day-old mutant flies, but a significant loss of DA neurons in PPL1 and PPM1/2 (but not PPM3 clusters) was recorded when these flies are 60-day of age. This result suggests that a significant length of time is required for LRRK2 mutants to manifest their neurotoxicity in the fly, a phenomenon that is consistent with the typically late-onset phenotype of LRRK2-linked disease. Conceivably, this time-dependent degeneration may explain why Lee et al did not observe any loss of DA neurons in 30-day-old flies expressing orthologous LRRK2 R1441C mutant [154], although the speculation remains to be proven. Importantly, Imai et al identified the eukaryotic initiation factor 4E-binding protein (4E-BP) as a substrate for LRRK2. Notably, phosphorylation of 4E-BP relieves its inhibition on protein translation, an energy-demanding process that could be detrimental when unregulated especially in times of stress. Consistent with this, *Drosophila* expressing LRRK2 mutants exhibit enhanced sensitivities towards oxidative stress agents whereas flies that are deficient in LRRK2 expression are resistant. These results offer a novel pathogenic mechanism by which LRRK2 mutations could result in an age-dependent loss of DA neurons.

Another possible mechanism of LRRK2-induced neurotoxicity comes from an elegant study performed in *C. elegans* by Sakaguchi et al [156]. The investigators demonstrated that deletion of orthologous LRRK2 gene in *C. elegans* significantly affects the transport or sorting of synaptic vesicles (SVs) in the worm's neurons. Normally, SVs are localized to the axonal regions but not to dendritic processes. In LRRK2-deficient worms, this polarized localization of SV proteins is lost and SV proteins are found in both presynaptic as well as dendritic endings of neurons. The axonal-dendritic polarity is however unaffected in transgenic worms expressing wild type LRRK2. Interestingly, the aberrant SV localization in LRRK2-deficient worms could be rescued by the introduction of an orthologous disease-associated LRRK2 mutant (that corresponds to the human LRRK2 I2020T mutation) but not by a kinase-inactive form of LRRK2. Together, these results demonstrate a non-dispensable role for LRRK2 in the polarized sorting of SV proteins to the axons and at the same time, suggest a mechanism by which loss of LRRK2 function could also result in neuronal dysfunction.

### **Concluding remarks**

Although the discovery of familial PD-linked genes have provided the opportunity to develop genetic models that hold promise to recapitulate the chronic nature of the disease, it is rather disappointing that virtually none of the genetic mouse models of PD generated thus far has managed to replicate the disease pathology and progression faithfully. Further, conflicting observations amongst different groups regarding the phenotypes displayed by the various models are not infrequent. However, in retrospect, it might be a tall order for animals living in controlled environment to

recapitulate the various features of a complex disease that typically requires several decades to surface in humans, even in individuals with overt PD-linked gene mutations. Perhaps a second (or multiple) hit present in the environment at large is needed to promote overt DA degeneration in these genetically-manipulated animals, as suggested by the accelerated degeneration of DA neurons in DJ1  $-/-$  mice in response to MPTP intoxication [141]. Surprisingly, the most dramatic phenotype is observed in *Drosophila* PD models. Indeed, it remain intriguing that some fly models of PD could reproduce the salient features of the human disease, including progressive degeneration of select subsets of DA neurons and associated locomotion deficits. However, it is important to note that several major abnormalities outside the fly's nervous system occur just as frequently, if not more. Thus, not a single model to date represents a perfect replica of the disease. Whether mouse models of LRRK2-induced parkinsonism would eventually turn out to be an ideal genetic model of the disease remains to be seen. However, as mentioned earlier, the disease mechanism of LRRK2 dysfunction is not entirely clear at this moment. Although a dominant gain of function mechanism is suspected, a dominant-negative or haploinsufficiency mechanism is also plausible, particularly in view of the abnormal phenotype in LRRK2-deficient worms. How LRRK2-linked parkinsonism should be best modelled is therefore academic. Nonetheless, it is important to recognize that existing models have collectively provided several important insights into the pathogenesis of PD.

At least with loss-of-function PD mouse models, abnormality in nigrostriatal DA neurotransmission seems to be a frequent, albeit subtle, phenotypic characteristic that accompanies gene dysfunction. Both PINK1  $-/-$  and DJ-1  $-/-$  mice, together with some mutant synuclein-expressing mice, have been described to exhibit reduced DA release,



while the reverse is true for parkin mutant mice (Table 2) as well as mice ablated of  $\alpha$ -synuclein [157]. Whether defects in DA release or reuptake mechanism precede and/or contribute to DA degeneration is unclear at present, but this line of investigations certainly warrants attention, especially in view of the association of the phenomenon with not one but four PD-linked genes. A caveat is that the above phenomena are not consistently observed amongst different groups (Table 2). On the other hand, *Drosophila* PD models have provided significant insights into the relationship between recessive PD-linked genes and mitochondrial function. The fact that mitochondrial pathology in the fly's flight muscle is a major feature of *parkin* and *pink1* mutant flies would suggest a non-trivial role for these two proteins in maintaining mitochondrial homeostasis. Further, DJ-1 also appears to participate directly or indirectly in ensuring proper mitochondrial function. Moreover, mitochondrial dysfunction is widely thought to underlie sporadic PD pathogenesis. The obvious challenge now is to translate the findings obtained in flight muscles of flies into neuronal cells, and to address alongside whether and how mitochondrial impairment affects nigrostriatal DA neurotransmission. At the same time, it would be important to elucidate the nature of interaction(s) amongst the different PD-linked gene products associated with mitochondrial homeostasis. The clarifications of these issues could provide clues to facilitate the generation of a more representative genetic model of PD, and thereby the development and assessment of therapeutic strategies.

In conclusion, despite the current lack of a representative model of PD that accurately recapitulates the progressive process as well as the nature and distribution of the human pathology, what we already have from existing genetic models of PD is a

wealth of information waiting to be exploited. Rather than over-emphasizing on the lack of robust PD phenotype in these models, it would be worthwhile to examine more closely the subtle, but nonetheless, recurring phenotypes of the various models that may represent important pre-clinical features of the disease.

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**Figure legend**

**Figure 1. DA system in *Drosophila* and *C. elegans*.** (A) Schematic figure (*top panels*) and anti-TH-immunostained images (*bottom panels*) showing the distribution of various DA neuronal clusters in the fly brain. PAL, protocerebral anterior lateral; PAM, paired anterolateral medial; PPM, protocerebral posterior medial; protocerebral posterior lateral. (B) Schematic depiction of DA neuronal subsets in the worm. CEP, cephalic cells (2 dorsal and 2 ventral); ADE, anterior deirids (2 bilateral); PDE, posterior dierids (2 bilateral).

Table 1:  $\alpha$ -Synuclein mouse models

Transgene	Promoter	Nigral Cell Loss	Protein Inclusions	Motor Impairment	Additional features	Ref.
WT	PDGF- $\beta$	-	+	+	Inclusions are non-fibrillar.	[31]
WT	Thy-1	-	+	+	Also noted motor neuron and brain stem pathology in these mice.	[34]
A53T		-	+	+		
WT	Prp	-	-	-	No obvious neurological dysfunction despite high levels of transgene expression	[32,33]
A30P		-	-	-		
A53T	Prp	-	+	+	Pathology abundant in brain stem and other brain areas. Truncated $\alpha$ -synuclein species observed.	[32,33]
A30P	Prp	-	-	+	Widespread CNS gliosis. Truncated $\alpha$ -synuclein species observed.	[35]
WT	TH	-	-	n.d.	Levels of striatal DA unchanged in transgenic animals compared to control littermates.	[36]
A53T or A30P		-	-	n.d.		
WT	TH	-	-	-	Significant reduction in locomotor activity compared to control mice following MPTP treatment.	[37]
A53T/A30P	TH	+	-	+	Also exhibit age-related reduction in DA level.	[37,38]
WT (1-120)	TH	-	+	+	Inclusions in SN and olfactory bulb. Transgenic mice lack endogenous $\alpha$ -synuclein. Reduced striatal DA.	[39]
A53T (1-130)	TH	+	-	+	Selective but non-progressive loss of A9 (but not A10) DA cells. Occurs during embryogenesis.	[40]
WT	CaMKII $\alpha$	+/-	-	+	Conditional model. Mice exhibit modest SNr degeneration and cognitive impairments.	[46]

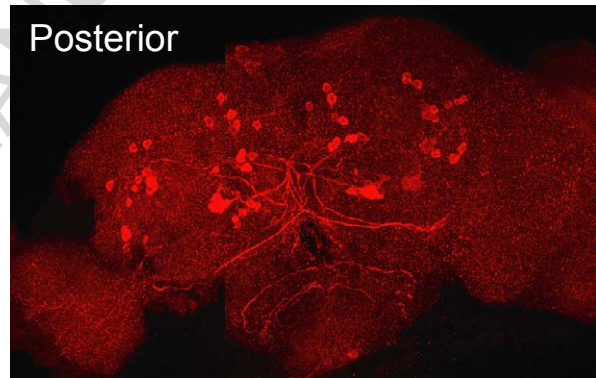
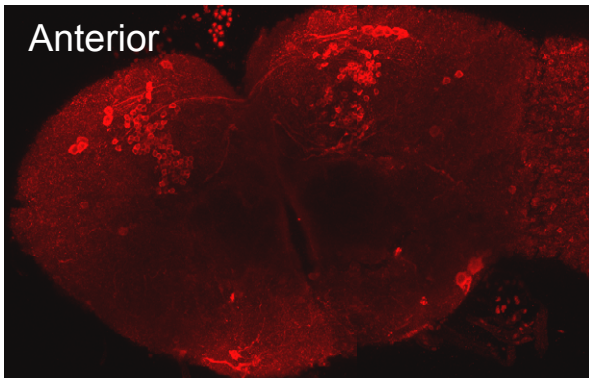
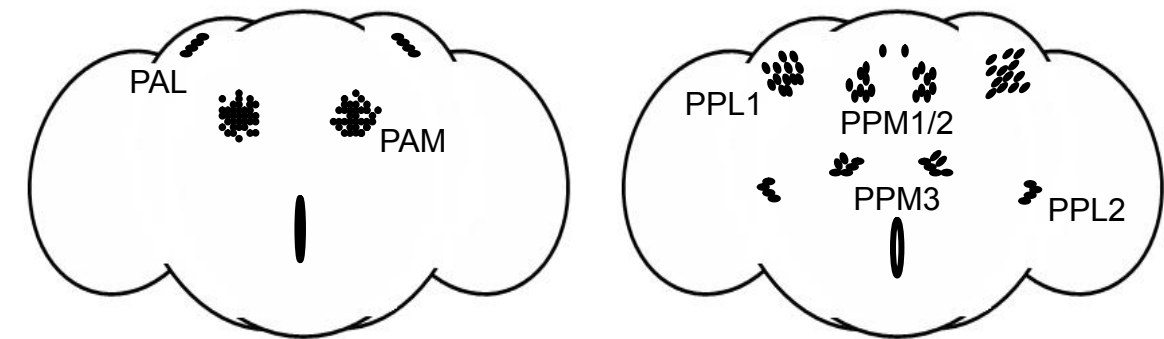
n.d. not described

**Table 2: Mouse models of autosomal recessive parkinsonism**

<b>Ablated Gene</b>	<b>Exon deleted</b>	<b>Nigral Cell Loss</b>	<b>Protein Inclusions</b>	<b>Motor Impairment</b>	<b>Additional features</b>	<b>Ref.</b>
Parkin	3	-	-	-	Increased extracellular DA level.	[77]
Parkin	3	-	-	+	Reduced DAT and VMAT levels. Cognitive deficits.	[78]
Parkin	2	-	-	-	Normal neurochemistry. No robust phenotype.	[79]
Parkin	7	-	-	-	Loss of LC neurons. Reduced acoustic startle.	[80]
PINK1	4-7	-	-	-	Reduced evoked DA release. Impaired mitochondrial respiration in striatum.	[123, 124]
DJ-1	2	-	-	+	Increased DA reuptake. Reduced evoked DA release.	[132, 133]
DJ-1	1-5	-	-	+	Increased DA reuptake.	[134]
DJ-1	2	-	-	+	No difference in evoked DA release compared to WT.	[135]
DJ-1	7*	-	n.d.	+/-	Enhanced DAT levels in presynaptic membranes	[136]
DJ-1	2-3	-	n.d.	-	Increased mitochondrial H2O2 production	[137]
DJ-1	3-5	-	n.d.	-	Hypolocomotion observed only in response to amphetamine or MPTP challenge.	[141]

n.d. not described; \*translation blocked

A



B

