Parkinson’s disease (PD), the second most common neurodegenerative disorder, is characterized by a loss of dopaminergic neurons in the substantia nigra, as well as in other brain areas. The currently available dopamine replacement therapy provides merely symptomatic benefit and is ineffective because habituation and side effects arise relatively quickly. Studying the genetic forms of PD in animal models provides novel insight that allows targeting of specific aspects of this heterogenic disease more specifically. Among others, two important cellular deficits are associated with PD; these deficits relate to (1) synaptic transmission and vesicle trafficking, and (2) mitochondrial function, relating respectively to the dominant and recessive mutations in PD-causing genes. With increased knowledge of PD, the possibility of identifying an efficient, long-lasting treatment is becoming more conceivable, but this can only be done with an increased knowledge of the specific affected cellular mechanisms. This review discusses how discoveries in animal models of PD have clarified the therapeutic potential of pathways disrupted in PD, with a specific focus on synaptic transmission, vesicle trafficking, and mitochondrial function.

Keywords: Parkinson’s disease; animal models; therapeutic strategies; synaptic transmission; mitochondria

Introduction

Parkinson’s disease (PD) is a neurological disorder that affects an estimated 6 million patients worldwide. As worldwide life expectancy rises, the incidence of neurodegenerative diseases intimately related to the aging process, including PD, continues to grow. This leads to increased urgency to identify improved therapies that delay the progression and mitigate the disability associated with these scourges. While aging plays an important role in PD onset, other factors, such as environmental insults and genetic alterations, are known to cause this neurodegenerative disease. The most striking symptoms in PD are the motor problems, including tremor and bradykinesia. However, symptoms such as sleep pattern deficits, constipation, and memory loss are common in PD patients as well. Therefore, PD is a heterogeneous disease caused by a multitude of factors that, in an unknown fashion, result in a variety of symptoms.

The pathological hallmark of PD is the loss of dopaminergic neurons, which is linked with the motor deficits of this disease. Available treatment focuses on increasing dopamine levels in affected brain areas to postpone and minimize the symptoms caused by degeneration of dopaminergic neurons. This strategy leads to various side effects and can be ineffective, as it may lead to habituation or overt dopaminergic neuron death. In addition, many other brain areas are affected, potentially also explaining several of the nonmotor symptoms. Moreover, the existing therapeutic strategies are focused on the alleviation of symptoms, as the etiology of the disease remains largely unexplained. Given the increased incidence of PD and the lack of effective treatments, novel therapeutic approaches that target the molecular defects in PD are urgently needed.

The majority of PD cases are sporadic (90%), with an unknown cause; yet, risk factors have been
Table 1. Summary of Parkinson’s disease–associated genes, animal models, and their phenotypes

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Gene locus</th>
<th>Inheritance</th>
<th>Gene</th>
<th>Presence of Lewy bodies</th>
<th>Dopaminergic neuron loss</th>
<th>Motor deficits</th>
<th>Presence of Lewy bodies</th>
<th>Dopaminergic neuron loss</th>
<th>Motor deficits</th>
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<td>x</td>
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<td>+</td>
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<td>–</td>
<td>x</td>
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<td>–</td>
<td>+/–</td>
<td>+</td>
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<td>PINK1</td>
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<td>–</td>
<td>x</td>
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<td>+/–</td>
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<td>+</td>
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<td>+/–</td>
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Note: The existence of a rodent or fruit fly model is marked with an “x.” The presence of PD-related phenotypes was classified in the existent animal models: +, majority of the reports confirm the presence of the phenotype; +/–, no consensus in the field; –, majority of the reports confirm the absence of the phenotype; and the absence of any of these signs shows an unreported phenotype.

identified in these forms of PD, including age, drug abuse, and environmental factors, such as exposure to pesticides.8,7 The remaining fraction (10%) is familial, and several causative genes have been identified (Table 1).8–10 Interestingly, the familial cases of PD have a strong resemblance to the sporadic ones, suggesting that the affected pathways may be similar or overlapping. In line with this idea, genome-wide association studies reveal some genes linked to familial forms of PD that also behave as risk factors in sporadic forms of the disease.11 The discovery of PD-related genes has been essential to the establishment of the affected pathways in this disease. In particular, there appear to be differences in the molecular events affected by the genes causing dominant forms of the disease and those causing recessive forms. We propose that a rough line can be drawn between genes that cause PD because of dominant mutations that affect vesicle trafficking and synaptic transmission, and genes that are associated...
with recessive mutations and associated with mitochondrial dysfunction (and indirectly also synaptic transmission). This division may not only allow the creation of optimized PD animal models for different types of PD but also optimized treatment on the basis of specific mechanisms. A better understanding of how deficiencies in these pathways cause PD could lead to a better understanding of both familial and sporadic PD.

This review focuses on the translation of available knowledge on PD to the creation of suitable animal models that allow the understanding of the mechanisms of disease. As these mechanisms still remain largely unknown, the selection of animal models can only depend on the resemblance of neurological lesions observed in PD patients to data from animal models’ phenotypes upon comparable genetic or environmental insult. Additionally, a good animal model should show responses to medications similar to those of PD patients. Depending on the mutations being modeled, a good animal model may show impaired vesicle trafficking and/or mitochondrial dysfunction and present symptoms and alterations reminiscent of those observed in PD patients suffering from similar mutations. Disappointingly, such a flawless model does not exist at this point. Scientists therefore use an array of chemical, genetic, and environmental insults that promote some of the pathogenic events of PD in order to study specific mechanisms of this disease. In doing so, several defects can be reproduced in animal models, and by studying the mechanisms underlying these symptoms pieces of the mechanistic puzzle can be put together. The use of both toxin-based and genetic-based models to study PD is relevant for aspects of both sporadic and genetic forms of PD. Whether PD is caused by a cell- or non-cell–autonomous mechanism remains a subject of controversy in the PD field. PD is characterized by a loss of dopaminergic neurons in a specific area of the brain, but whether these neurons are more sensitive to PD gene mutations or environmental stresses, or whether dopaminergic neuron loss is a consequence of a general dysfunction in the central nervous system (or even outside the central nervous system) is still unknown. The available data on current animal models are insufficient to address these questions. The fact that none of the current models show the full spectrum of symptoms or respond similarly to drugs as PD patients precludes firm conclusions on the exact origin of the disease.

The current models do, however, allow the study of individual cellular mechanisms that are impaired in PD patients, including, for example, dopamine release and mitochondrial dysfunction. The absence of the complete range of phenotypes could originate from (1) the absence of a homologous gene in the animal model; (2) a lack of a similar neuronal circuitry structure in the different species used as animal models; or (3) differences in the cellular function of the disease-associated protein in human versus animal models or cell types. In our view, PD research can mostly benefit from animal models in the discovery of new affected cellular processes (that can be conserved in those animals) and in testing new therapeutic approaches in well-characterized processes.

We will summarize the phenotypes and the research potential of relevant animal models in PD, discussing the genetic ones that show synaptic dysfunction, the models that allow the study of impaired mitochondria, and newly discovered genes that may open avenues for creating novel animal models. Assuming PD subgroups differentially respond to specific therapeutics, this stratification is relevant to bridging the affected pathway—in each patient with the pharmacological targets that can modulate the pathway. We also propose novel approaches to the discovery of new targeted therapies with the intention of preventing or delaying PD.

Synaptic transmission defects in Parkinson’s disease

One of the hallmarks of PD is the marked reduction of dopamine release from the substantia nigra, leading to the characteristic motor defects. Mutations in the gene SNCA, which encodes for α-synuclein (α-syn), are one of the causes of autosomal dominant PD. Present research on α-syn supports a role of this protein at synaptic terminals and, more specifically, in neurotransmitter release. LRRK2 mutations (encoding leucine-rich repeat kinase 2) similarly result in autosomal dominant familial PD, but they are also found in patients suffering from sporadic PD. Recently, studies have shown that LRRK2 is involved in endocytosis and synaptic vesicle trafficking. These findings suggest that mutations in genes involved in...
autosomal dominant PD mutations, such as SNCA and LRRK2, may center on defects in endocytosis and vesicle trafficking in selected neuronal clusters. In fact, reduced dopaminergic release is a commonly observed feature in PD and has been reported in the genetic models of SNCA (Snca in the mouse) and LRRK2 (Lrrk, in the mouse). Research on this defective synaptic mechanism may be of relevance to the development of novel therapeutic approaches for the patients with dominant mutations in PD-related genes. Additionally, the treatment of patients with sporadic PD with these synaptic defects may also be improved by research in genetic animal models based on SNCA and LRRK2.

α-Synuclein

SNCA was the first gene identified to be causatively associated with PD. α-Syn is found in aggregated form in Lewy bodies. Hence, initial PD research focused on α-syn aggregation, but the physiological and pathological roles of this protein remain elusive. α-Syn aggregation can result in either a loss of protein activity or the accumulation of toxic species in the cell. The relation between protein aggregation and cell death is still controversial. Indeed, it has been proposed that the intermediary form of the aggregated α-syn (prefibrillar, soluble oligomeric species) is the main cause of cell death, not the aggregated α-syn in Lewy bodies. Lewy bodies can be composed of more than 70 proteins, including α-syn, and are visually characterized by the presence of a dense core surrounded by a halo, composed of granular and fibrillar components. However, Lewy bodies have a heterogeneous structure, depending on neuron type and protein composition. Given this wide definition, researchers sometimes characterize protein aggregates as Lewy bodies, even if they do not present all of the characteristic features.

Studies on α-syn support a role for this protein in synaptic terminals and, more specifically, in neurotransmitter release and synaptic vesicle reclustering (Fig. 1). Patients with rare autosomal dominant familial mutations in SNCA have been identified, highlighting the association of this gene in PD. In addition, point mutations (A53T, A30P, and E46K), duplications, and triplications of SNCA have been shown to be causative for the disease. Since triplication of SNCA causes earlier and more severe symptoms than duplications, it was concluded that α-syn overexpression affects the neurons in a dosage-dependent manner. It is therefore important to understand the molecular and cellular consequences of α-syn abnormalities for neuronal function.

α-Syn is a 140-amino acid presynaptic protein that contains three domains: (1) a lipid binding motif, (2) a central hydrophobic region, and (3) a highly negative charged C terminus. The first two domains are predicted to promote lipid binding. Upon neuronal activity, α-syn transiently binds to synaptic vesicles, which stabilizes and protects them from premature fusion caused by vesicle curvature stress. Thus, overexpression of α-syn leads to the accumulation of synaptic vesicles owing to inefficient release that ultimately results in deficient synaptic transmission. The mutation A30P in α-syn results in reduced binding of α-syn to the membrane, thought to lead to defective vesicle organization close to the active zone, resulting in decreased vesicle release. α-Syn directly binds to the SNARE proteins and promotes SNARE-complex assembly. The effect of α-syn accumulation at the synapse causes the redistribution of the SNARE complex involved in exocytosis. Conversely, increased expression of α-syn inhibits synaptic vesicle reclustering after endocytosis, also contributing to less neurotransmitter release by reducing the number of available synaptic vesicles. Additionally, the A30P and A53T α-syn mutations are thought to promote vesicle permeabilization and leakage of vesicular components, again impairing synaptic transmission by reducing the level of neurotransmitters inside synaptic vesicles. These findings suggest that α-syn can regulate the functionality of the synaptic vesicle pool, which in turn would influence synaptic transmission and plasticity (Fig. 1).

The relevance of α-syn in cell survival and synaptic function has been highlighted with several transgenic animal models of α-syn. Several transgenic mouse lines, expressing wild-type or mutated Snca under the control of various promoters, have been created, leading to a wide range of phenotypes, the severity of which could potentially represent different stages of the disease.

The α-synuclein models

The overexpression of Snca in mice using pan-neuronal promoters leads to defective mitochondria, defective motor activity, and progressive
nigrostriatal neurodegeneration that is accompanied by synaptic dysfunction.\textsuperscript{46,47} Overexpression of \textit{Snca} in the nervous system of mice, using the pan-neuronal \textit{Thy1}, \textit{Prh1}, or \textit{Pdgfa} promoters,\textsuperscript{48} results in \(\alpha\)-syn aggregation and accumulation in neuronal cell bodies and at synapses.\textsuperscript{49} These mice closely recapitulate several pathogenic features resembling PD.

In addition, \textit{Drosophila melanogaster} models have also contributed to understanding the role of \(\alpha\)-syn in PD pathology. While wild-type fruit flies do not express \(\alpha\)-syn, ectopic neuronal expression of this protein appears to promote progressive dopaminergic neuron loss, with widespread Lewy-body–like inclusions that lead to impaired motor function.\textsuperscript{50–52}

The specific mechanism by which \(\alpha\)-syn affects dopaminergic nigrostriatal neurons without damaging many of the remaining neurons is still elusive. If the disease originates specifically in dopaminergic neurons, the restriction of wild-type and mutant \textit{Snca} overexpression to dopaminergic neurons, using the tyrosine hydroxylase (\textit{Th}) promoter, would reveal some, if not all, of the phenotypes of the mice with \textit{Snca} overexpression in the whole brain. However, overexpression of \textit{Snca} under control of the \textit{Th} promoter does not result in the characteristic deficits observed in PD patients: the mice do not exhibit Lewy bodies, decreased dopamine levels, or motor defects. Such defects are visible when \textit{Snca} is expressed under a pan-neuronal promoter.\textsuperscript{53,54} These data led to the speculation that PD does not originate specifically in dopaminergic neurons and led to the proposition that these neurons are affected only during the progression of the disease.

To further test this hypothesis, other strategies were taken to promote \(\alpha\)-syn aggregation and degeneration of dopaminergic neurons. Since the C-terminus of \(\alpha\)-syn inhibits its aggregation\textsuperscript{55} and a C-terminal–truncated form of \(\alpha\)-syn was found in postmortem brains of PD patients,\textsuperscript{56} mice overexpressing the C-terminal–truncated form of \(\alpha\)-syn under the \textit{Th} promoter were created. These mice have an accelerated aggregation state without neuronal death,\textsuperscript{57} and thus they are considered to be a good model for the initial stages of PD. Synaptic dysfunction without cell death suggests a “die back” mechanism, where dysfunctional synapses precede neuronal death. The effects of overexpression of C-terminal–truncated \(\alpha\)-syn primarily in synapses rather than the cell bodies correlate with the fact that PD patients display only 30% of dopaminergic neuron loss at the time of the first motor symptoms, when a massive loss of released synaptic dopamine is observed.\textsuperscript{56} This link suggests that synapses might be a good primary target for therapeutics, with the hope that prevention of synaptic loss can avoid neuronal death. At the same time, this model presents dopaminergic system dysfunction as an important trigger to some of the PD symptoms.

While the role of \(\alpha\)-syn inside neurons is studied intensively, its extracellular function has been much more neglected. In fact, aggregated \(\alpha\)-syn is released

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Synaptic transmission is impaired upon \(\alpha\)-syn overexpression. (A) Fluorescence intensity (of VGLUT1-pHluorin) increases upon synaptic vesicle exocytosis. Overexpression of \(\alpha\)-syn disrupts the exocytotic process by lowering the amount of released neurotransmitter. (B) Synaptic vesicle reclustering after endocytosis is impaired if \(\alpha\)-syn is overexpressed. After a stimulus, the number of recovered synaptic vesicle containing GFP-VGLUT1 is decreased under \(\alpha\)-syn overexpression conditions. This occurs without defects in the first steps of vesicle formation, showing a defect in synaptic vesicle reclustering following endocytosis. Figure reprinted from Ref. 18.}
\end{figure}
Brain homogenates of old mice expressing A53T mutant -syn (that show motor defects and accumulation of -syn) were intracerebrally inoculated in young mice from the same line without a phenotype, triggering motor defects in these young animals. For the first time, a prion-like mechanism was observed in an animal model of PD. More recently, injection of synthetic -syn fibrils in the striatum of wild-type mice resulted in cell-to-cell propagation, and aggregation of this protein only occurs in those brain regions that anatomically connect to the site of injection, such as the cortex and olfactory bulb. This local injection of synthetic -syn leads to reduced dopamine release and motor deficits. These recent data on the transmission of -syn are paving the way for novel research lines that propose to incorporate both intracellular and extracellular -syn functions in the context of PD.

Novel PD animal models have been created to allow the fine temporal and spatial modulation of -syn. As opposed to transgenic insertion, viral vector approaches allow for the targeted and differential expression of the desired transgene in a restricted neuronal population at a certain time. Infection of the substantia nigra of adult animals is achievable using a single injection. The use of viral vectors constitutes a flexible means to rapidly produce mutant proteins in order to study their effects in a restricted neuronal population and study their interactions with environmental triggers or proposed treatments. Viral vector production is often more straightforward and faster than the production of transgenic lines. One crucial advantage is the possibility of using the contralateral hemisphere as an internal control. In one study, rats expressing wild-type and A30P or A53T mutant -syn after infection with a lentivirus resulted in a specific loss of dopaminergic neurons ipsilaterally, leaving the contralateral neurons intact. The most obvious drawback of this technique is the requirement of an intracerebral injection with all the associated variability and intensive animal handling. While this model allows the study of an abrupt perturbation in protein levels in an adult brain and circumvents problems with developmental lethality or deleterious transgenes and compensatory mechanisms, it requires individual manipulations of each animal in order to inject the virus. Despite these disadvantages, several animal models, virus types, ages, and sites of injections were proposed with encouraging results.

A novel, recently published technique based on the intranasal uptake of -syn could solve the problem of the intense animal handling. The necessity of extensive animal handling, as in this invasive protocol, promotes animal stress, which can alter the effect of the treatment. Additionally, since animals need to be treated individually, the delivery site of the virus cannot be exactly replicated, and technical variability tends to increase. Intranasal administration of -syn aggregates results in motor defects only after 14 days, without a prolonged, stressful, and invasive procedure. These defects are caused by a reduction of dopamine levels and its metabolites, mainly occurring in substantia nigra neurons. Although this model allows the study of PD in adult animals without affecting their development by overexpression of -syn at early developmental stages, this novel model requires further characterization and validation. In the future, this acute model of -synucleinopathy can be used to trigger the characteristic substantia nigra dopaminergic neurodegeneration in adult animals in the presence of a specific drug or of a specific mutant background. This would allow the discovery of previously unstudied pathways that can be targeted pharmacologically, as well as the establishment of high-throughput screening for drugs that prevent or delay PD symptoms.

In summary, the novel PD animal model based on the intranasal administration of -syn aggregates, in addition to the ones obtained by viral vector injection, is an alternative in PD research to the classical transgenic animal models. Advantages include the modulation of -syn at a specific time and location, and the possibility of drug treatments and the presence of mutations in the animal background before the expression of -syn. While -syn animal models were the first ones to be studied, they still have enormous potential to enable new discoveries in the PD-affected pathways. Although the mechanisms of toxicity of -syn oligomers are still under debate, genetic overexpression of Snc results in dopaminergic neuron death that is associated with motor deficits. While animals that overexpress Snc show variable phenotype severity...
Figure 2. Endocytic defects in loss of LRRK2 function. (A) Traces of current clamp recordings in high external calcium of the excitatory junction potential (EJP). Loss of LRRK2 fails to maintain release during intense stimulation (10 Hz). For comparison, the control data are added in gray behind the LRRK2 data. (B) Labeling of FM1–43, a lipophilic dye that, when inserted in the membrane, becomes fluorescent and is internalized into newly formed synaptic vesicles upon nerve stimulation. Loss of LRRK shows defects in synaptic vesicle formation. Figure reprinted from Ref. 20.

and are not able to reproduce the whole spectrum of PD features, they still are the most studied model of PD.

LRRK2

PD can also be caused by autosomal dominant mutations in LRRK2. Mutations in this gene are the most common genetic mutations that cause PD (3–40%)\(^{19,31}\) and mutations in LRRK2 have also been found in sporadic PD patients,\(^{69}\) possibly providing an etiologic link between these sporadic and genetic forms of PD. Some mutations in LRRK2 are clearly causative for the disease while others are a common risk factor with a population-dependent penetrance.\(^{70}\) LRRK2 harbors several protein domains, including a GTPase and kinase domain.\(^{19}\) While LRRK2 has been implicated in a range of processes, recent evidence suggests a role for LRRK2 in synaptic vesicle trafficking and endocytosis (Fig. 2),\(^{20–22,24}\) processes where extracellular material and a portion of the plasma membrane invaginate into the cell to form membrane-bound vesicles.\(^{71}\) Endocytosis plays an important role in many cellular processes, including synaptic transmission,\(^{72}\) but how this process is involved in neurodegenerative disease remains elusive.

The most common mutation that accounts for 3–10% of familial forms of PD is the G2019S mutation, which, as most LRRK2 mutations, is localized in the kinase domain, but unlike most other LRRK2 mutations, the G2019S mutation is responsible for upregulation of LRRK2 kinase activity.\(^{8,73}\) G2019S causes PD that is strikingly similar to the typical late-onset PD with Lewy body formation.\(^{74,75}\) Another common mutation, R1441G, was used to create the first Lrrk2 transgenic mouse that displays several key characteristics of PD, including slowness of movement, reduced dopamine release, and responsiveness to levodopa treatment.\(^{76}\) As PD-related mutations are considered as gain-of-function mutations, mice overexpressing wild-type LRRK2 and G2019S LRRK2 were created. While mice overexpressing wild-type LRRK2 show elevated dopamine release and increased motor performance, mice overexpressing G2019S LRRK2 display an age-dependent reduction of dopamine release; however, no dopaminergic neuron loss was observed.\(^{77}\) In contrast, another study showed dopaminergic neuron loss in mice expressing G2019S mutant LRRK2 but here, motor defects were lacking.\(^{78}\) In sum, the available data indicate that LRRK2 activity is critical for normal neuronal function as well as in controlling dopamine levels, and mutations in this protein suggest a gain of function that can result in symptoms reminiscent of PD-related motor defects or reduced dopaminergic release.

A loss of LRRK function in flies, who carry only one ortholog of LRRK, appears to cause the degeneration of some dopaminergic neurons and motor symptoms.\(^{79}\) Furthermore, flies expressing G2019S-mutated human LRRK2 also appear to display some dopaminergic neuron loss.\(^{80}\) Data from fly studies also revealed that LRRK may play a direct role at the synapse. LRRK functionally and genetically interacts with the synaptic protein endophilinA, which binds to membranes and controls the process of synaptic endocytosis. EndophilinA
how to use \( \alpha \)-syn and LRRK2 as pharmacological targets?

As stated before, \( \alpha \)-syn and LRRK2 have a wide range of functions in the cell. However, the most established model puts these proteins as regulators of synaptic transmission;\(^{20,24}\) this is of interest when aiming to alleviate PD symptoms. Given the close relation between \( \alpha \)-syn levels and its aggregate formation, an initial proposed strategy to prevent \( \alpha \)-syn aggregation is the reduction of its levels in the brain. Although the physiological role of \( \alpha \)-syn is not completely understood, mice with reduced levels of \( \alpha \)-syn seem to have only minor defects in synaptic transmission. Specifically, these mice have small defects in response to a prolonged train of repetitive stimulation, while basal transmission and the response to a brief train of high-frequency stimulation in nigrostriatal terminals remain normal.\(^{14,88}\) These mice are viable and fertile.\(^{14,88}\) While genetic therapy could aid in the decrease of \( \alpha \)-syn levels by shRNA inhibition of \( \alpha \)-syn mRNA translation, this technique is currently not in practice. Therefore, efforts are necessary to reveal the pathways that control \( \alpha \)-syn synthesis, with the aim to find a pharmacologic modulator of \( \alpha \)-syn production that can be tested in animals. It is currently not entirely clear if the full-blown aggregated \( \alpha \)-syn bodies are toxic rather than the oligomeric species, but reducing \( \alpha \)-syn load in the cell holds the advantage that the concentration of both aggregated species as well as oligomeric species may be reduced.

If the production of \( \alpha \)-syn cannot be controlled, and assuming aggregated \( \alpha \)-syn is toxic, strategies could turn to the removal of aggregated \( \alpha \)-syn from the cell. Owing to the direct relation between \( \alpha \)-syn levels and its aggregation potential, therapies that increase \( \alpha \)-syn clearance should prevent its aggregation and, if needed, remove the aggregated \( \alpha \)-syn in order to avoid dopaminergic neuron dysfunction. One approach is to promote \( \alpha \)-syn clearance by activating the ubiquitin-proteasome or the lysosomal systems; yet, both of these systems are affected in PD.\(^{89,90}\) Thus, finding targetable pathways that can reactivate these clearance systems is of relevance to PD therapeutic strategies. Conversely, increased autophagic flow, another process impaired in PD,\(^{91}\) could help the clearance of accumulated \( \alpha \)-syn. Rapamycin was tested as an autophagic promoter in \( \alpha \)-syn clearance\(^{92}\) but proved too toxic, leading to the development of small-molecule enhancers of rapamycin. Clinical trials with PD patients for \( \alpha \)-syn clearance by autophagy are also being considered.\(^{93}\) These approaches would result in a decreased intracellular accumulation of \( \alpha \)-syn, but much care will need to be taken that general increased autophagy or clearance mechanisms will not impede with other aspects of cellular neuronal function.

\( \alpha \)-Syn was shown to be released to the extracellular medium, where it can be toxic. Clearance of extracellular \( \alpha \)-syn could result in an alleviation of its deleterious effects in neurons. Antibodies that specifically recognize \( \alpha \)-syn help microglia to recognize and remove the extracellular \( \alpha \)-syn, thereby preventing the deleterious action on neighboring cells.\(^{94}\) However, it still needs to be clarified that the removed species are the deleterious ones and that essential \( \alpha \)-syn functions have not been removed, further disturbing the ratio between deleterious and functional \( \alpha \)-syn species even more. Additionally, the specificity of such antibodies is a main concern to avoid clearance of unwanted proteins. Although this technique could be highly effective, questions on its efficiency in the clearance of aggregated \( \alpha \)-syn in the brain arose because of the size of the antibody and the correspondent low permeability of the blood–brain barrier. However, recent reports suggest that peripheral administration of \( \alpha \)-syn antibodies
promoted the clearance of α-syn in the brain, increasing the deleterious α-syn. Conversely, an increased knowledge of the removed species and improved delivery methods need to be obtained before such new therapeutic applications can be used.

Similarly to the defective synaptic transmission observed upon α-syn aggregation, LRRK2 mutations might be associated both with a gain of toxic function and with a loss of synaptic coordination function. Thus, treatments should focus not only on the clearance of aggregated α-syn or modulating LRRK2 enzymatic activity but also on maintenance of the synaptic function. Current research is focused on identifying molecules that are able to modulate LRRK2 activity and thus prevent the synaptic dysfunction observed in PD patients with defective LRRK2. Although classical treatment with levodopa helps in the maintenance of synaptic function in the initial stages of the disease, it cannot help in avoiding synaptic loss at a later time. It is therefore necessary to identify novel therapeutics that combine an initial reestablishment of dopamine release with a prolonged stability of a functional synapse. Whether common drugs that target both LRRK2-affected pathways are α-syn-affected pathways remains to be seen, as the molecular mechanisms affected may be too divergent.

Mitochondrial function is involved in Parkinson’s disease

Mitochondria are abundant organelles that exert several important functions, including calcium homeostasis and energy conversion. Because of its function in energy production, mitochondria are often referred to as the powerhouse of the cell. The majority of ATP in neurons is generated via the aerobic metabolism at the electron transport chain (ETC). Adequate energy supply is critical for sustained neuronal function. Malfunction of different complexes of the mitochondrial ETC are associated with different neurodegenerative diseases. Over the last two decades, mutations in mitochondria-related genes, including parkin, PINK1, and DJ-1 have been identified to be causative for PD. While the number of PD patients with mutations in these genes that affect mitochondria is rather limited, mitochondrial defects are found in both genetic but also in sporadic cases of PD and thus a common underlying mechanism may be at play. Mitochondrial involvement is further supported by the observation that PD incidence is 2.5-fold higher when rotenone, a complex I inhibitor, was used in agricultural activities. Therefore, studying sporadic or toxin-induced (drugs or pesticides) and genetic animal models holds important value for the PD cases with mitochondrial abnormalities.

The toxin-induced models

The relation between mitochondria and PD was proposed in 1979 when the injection of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), the side product of the illegitimate synthesis of the heroin analog MPPP (1-methyl-4-phenyl-4-propionoxypiperidine), was found to result in Parkinsonism-like symptoms in humans. MPTP easily crosses the blood–brain barrier where it is oxidized in glial cells to obtain MPP+ (1-methyl-4-phenylpyridinium). MPP+ structure is very similar to dopamine and can therefore be taken up by dopaminergic cells, where it will exert its toxic effect by inactivating complex I of the ETC. MPTP is commonly used to model PD in primates and rodents in that the drug kills dopaminergic neurons, allowing researchers to study neuronal circuitry with reduced dopaminergic neuron involvement. In rodents, however, there is a more rapid turnover of this drug compared to primates. While rats do not exhibit any defects, specific strains of mice, including C57 BL/6J, display phenotypes similar to the defects observed in humans, including motor symptoms and a loss of dopaminergic neurons. An early report using MPTP in mice described the presence of a limited number of Lewy bodies, but later reports claimed not to observe any Lewy bodies. Hence, it seems that Lewy body inclusions are largely lacking in this model, similar to the lack of Lewy body accumulations in several genetic forms of the disease as well, and the major feature of MPTP is that it kills dopaminergic neurons.

Because of the reduced MPTP sensitivity in mice, higher doses need to be used to induce phenotypes; however, these high doses also induce a higher mortality rate. In contrast to the acute model where high doses of MPTP are injected, the chronic injection protocol does not induce mortality, and variability of induced phenotypes is very low. MPTP is also used in nonhuman primates, causing severe Parkinsonism with postural tremor, akinesia, and
a loss of dopaminergic neurons.\textsuperscript{115–117} Also here, the preference should be given to chronic injection protocols in order to include as many of the currently unknown mechanisms of the disease, which by definition is a chronic and progressive disorder. MPTP has proven its value in modeling aspects of PD in that it is a mitochondrial toxin and it creates conditions in animals where dopaminergic neurons die. The molecular events that result in death may or may not be similar to typical (mitochondrial) PD. Nonetheless, the drug holds the most potential when studying dopaminergic neuron circuits, but the value of MPTP when studying nondopaminergic neuron–related defects in PD may be more limited.\textsuperscript{106}

Similar to MPTP, the pesticide rotenone disrupts complex I function of mitochondria.\textsuperscript{118} Despite the lack of specificity of rotenone for dopaminergic neurons, it does result in a loss of dopaminergic neurons as well as in motor defects in different model species.\textsuperscript{119–122} Unlike MPTP, rotenone toxicity induces Lewy body formation in rats.\textsuperscript{120} Mice are less susceptible to rotenone-induced toxicity, and higher and multiple doses are required to induce dopaminergic neuron loss with motor symptoms.\textsuperscript{123}

The drawback of the use of this drug in rodents is the high variability of phenotypes because of different susceptibility in individual animals to rotenone toxicity, ranging from absence to complete loss of dopaminergic neurons.\textsuperscript{124–126} Rotenone is also used in the fruit fly to model PD. Specifically, flies treated with rotenone develop characteristic motor symptoms and degeneration of dopaminergic neurons. The motor symptoms, but not the dopaminergic neuron loss, can be alleviated with dopamine replacement therapy, suggesting that the motor symptoms arise from a shortage of dopamine levels.\textsuperscript{121} While PD is often associated with the loss of dopaminergic neurons, mitochondrial defects are observed in other brain areas and other tissues as well, including skeletal muscle and platelets.\textsuperscript{127,128}

A rotenone-based model may be of value because unlike MPTP, rotenone is a toxin that induces systemic mitochondrial defects, an event observed in PD. Both MPTP and rotenone have been important for the establishment of PD animal models. However, while they promote dopaminergic neuron death with associated motor impairments, their side effects and lack of specificity are the major drawbacks.

**The Parkin model**

Given that the chemical-induced animal models do not show all the classical phenotypes of PD, animal models based on PD patient gene mutations have been created as well. The majority of patients suffering from early-onset PD (20–40 years old)\textsuperscript{129} carry mutations in parkin (\textit{PARK2}),\textsuperscript{103} which encodes a cytosolic 465–amino acid protein that has a ubiquitin-like domain, 2 RING finger domains separated by an in-between RING domain.\textsuperscript{8} Parkin functions as an E3 ubiquitin ligase that ubiquitinates target proteins, both in the cytosol and in mitochondria.\textsuperscript{130,131} This ubiquitination leads labeled proteins and organelles (including mitochondria, as explained later) to be destined for degradation;\textsuperscript{132} however, how mutations in parkin result in the loss of dopaminergic neurons still remains elusive. Curiously, Parkin inactivation is observed with exposure to radical stress or posttranscriptional activations also observed in sporadic forms of PD.\textsuperscript{133} Therefore, a loss of Parkin enzymatic activity has been proposed to be critical for the onset of PD owing to the accumulation of Parkin targets and the decreased clearance of dysfunctional mitochondria and potentially other organelles and debris.

Initial mouse models for Parkin did not show loss of dopaminergic neurons or motor symptoms,\textsuperscript{134–136} suggesting that underlying mechanisms in Parkin-induced PD are protected against in mice or that a second trigger mechanism is required together with the loss of Parkin to produce PD-related deficits. This was supported by the observation that adult conditional knockout of \textit{parkin} results in a loss of dopaminergic neurons.\textsuperscript{137} Others have created BAC transgenic mice expressing a dominant negative form of Parkin. These mice exhibit motor deficits and a loss of dopaminergic neurons.\textsuperscript{138} In flies, loss of Parkin does induce motor symptoms and dopaminergic neuron loss, albeit with a various degree of severity from one animal to another.\textsuperscript{139–141} Loss of Parkin results in mitochondrial defects in flies, and abnormalities in mitochondrial morphology, reduced ATP levels, and defective mitochondrial membrane potential have been observed.\textsuperscript{140,142–145} While in \textit{parkin} knockout mice, reduced activity of complex I and IV of the ETC has been observed,\textsuperscript{146} in flies some reports show defects in ETC components while others do not, suggesting any effect at this level is marginal.\textsuperscript{144,147}
Parkin function has also been studied in *in vitro* models. The overexpression of Parkin in cultured cells has led to the identification of a function of Parkin in mitochondrial clearance. This process, termed *mitophagy*, is based on the relocalization of Parkin from the cytosol to the mitochondria, where it will ubiquitinate mitochondrial proteins, including mitofusin and the outer mitochondrial proteins TOM20, TOM40, and TOM70, to signal for degradation by the autophagosome. PINK1, a mitochondrial protein involved in PD (see later), is immediately partly proteolitically processed in healthy mitochondria; however, upon depolarization of mitochondria, PINK1 remains uncleaved and stabilizes at the mitochondrial membrane, providing a signal for Parkin to translocate to the mitochondria. Parkin is an E3 ligase that binds an E2 ubiquitin-conjugating enzyme to be functionally active. Rad6, a protein involved in mental retardation, is an E2 enzyme that binds to Parkin and is necessary for ubiquitination of mitochondrial proteins by Parkin, as ablation of Rad6 blocks the translocation of Parkin to the mitochondria. Recently, the de-ubiquitination enzymes USP15 and USP30 were identified as additional interactors of the mitophagy pathway, but some of these data are still conflicting. The idea here is that loss of the deubiquitinase improves mitochondrial function in Parkin mutants, suggesting that inhibition of such a deubiquitinase can overcome the defects at the level of mitophagy. Dissecting this pathway may result in a potential therapeutic target in the therapy of Parkin-dependent PD. However, it is important to stress that mitophagy is mostly observed following induction with a mitochondrial uncoupler to dissipate the membrane potential, and the functional relevance of this process on neuronal health and survival remains to be assessed. Furthermore, Parkin is not only involved in mitophagy, being a cytosolically localized protein it has several other nonmitochondrial targets as well that merit further study. Parkin removes cytosolic proteins by leading them to the proteasome system. Of special interest, Parkin ubiquitinates PARIS, a transcriptional repressor of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1a), that is involved in mitochondria biogenesis. Another substrate of Parkin is aminoacyl–tRNA synthetase complex interacting multifunctional protein-2 (AIMP2), overexpression of which in the mouse brain leads to a selective, age-dependent, progressive loss of dopaminergic neurons. This discovery of newly affected pathways by an absence of Parkin or inactivation of the protein are giving rise to novel ways to control PD progression, and may ultimately lead to new therapeutic avenues.

### The PINK1 model

The first familial PD cases that carry mutations in *PINK1* were identified in 2004. *PINK1* mutations occur in 1–9% of familial PD. *PINK1* is a Ser/Thr protein kinase that carries a mitochondrial targeting sequence. In mice, loss of *Pink1* does not cause dopaminergic neuron loss or motor symptoms. *Pink1* knockout mouse embryonic fibroblasts have mitochondrial defects, including reduced ATP levels and defective mitochondrial membrane potential. These defects can be explained by reduced complex I activity upon loss of *PINK1*. Loss of *PINK1* in *Drosophila* leads to reduced ATP levels and defective mitochondrial membrane potential, defects that are rescued by re-expressing wild-type human *PINK1* but not kinase dead *PINK1*, indicating evolutionary conservation of *PINK1* function and the involvement of kinase activity. Additionally, *Pink1* mutant flies also display motor symptoms and morphological abnormalities at the level of the mitochondrial network and cristae structure (Fig. 3). These phenotypes largely mimic those observed in *parkin* mutant flies, suggesting that these two proteins act in a common pathway. Moreover, overexpression of Parkin can partly rescue aspects of the *PINK1* loss–induced phenotypes in flies, while in reverse, overexpression of *Pink1* does not rescue *parkin* mutant phenotypes, suggesting that, in this capacity, *PINK1* functions upstream of *Parkin*. Assuming the functions of *PINK1* and *Parkin* are evolutionary conserved, these data are difficult to reconcile with a role for *PINK1* in Parkin recruitment because in this model, the absence of *PINK1* would preclude Parkin recruitment, even when the protein is overexpressed. Hence, further work is needed to explain and reconcile the genetic data with the *in vitro* evidence.

*Pink1* mutant *Drosophila* also display reduced complex I activity and this defect appears to occur independent and in parallel to other mitochondrial defects, such as remodeling or potentially also mitophagy. First, the defects in the ETC of *PINK1*
mutants are specific and other parts of the chain are still intact. A defect in mitophagy would be expected to hamper the function of different mitochondrial components at once. Second, in fruit flies the promotion of mitochondrial fission, a prerequisite for the induction of mitophagy, does not (or at least not strongly) rescue the reduced complex I deficiency in Pink1 mutants, suggesting that even when mitophagy is promoted in the absence of PINK1, the enzymatic defect at the level of complex I still remains. Finally, expression of a yeast protein, Ndi1, that bypasses functions of complex I by shuttling electrons from NADH to ubiquinone directly, rescues the mitochondrial defects in Pink1 mutant flies, but not in parkin mutant flies. Hence, genetic conditions that rescue PINK1 deficiency but not Parkin deficiency exist, suggesting that the mitochondrial defects in these mutants may have, in part, also a different origin. This idea was more recently further supported by the observation that the expression of the activated form of Ret, the signaling receptor of the glial cell line–derived neurotrophic factor, can rescue mitochondrial defects in Pink1 mutant flies while it fails to do so in parkin mutant flies. Furthermore, overexpression of this activated Ret protein specifically rescues the complex I defects in these Pink1 mutant flies, suggesting complex I function is an important aspect of the PINK1 loss-of-function phenotype. Providing molecular insight into the PINK1-induced defects, recent work showed that NdufA10, a complex I subunit, fails to be phosphorylated in Pink1 mutants and this phosphorylation event is critical to efficiently couple electron transport between complex I and ubiquinone, thus maintaining proper mitochondrial function. Interestingly, targeting these ETC defects observed in Pink1 mutants may prove to be a prosperous therapeutic strategy as, in fruit flies, infrared light–induced stimulation of the ETC or feeding vitamin K2, a compound that functions as an alternative electron carrier molecule in mitochondria, is capable of rescuing many of the defects observed in Pink1 mutant animals (discussed in more detail below). These and several
other therapeutic targets should now be evaluated further and potentially also tested for additive effects in order to yield better, more complete rescue of the defects.161

The DJ-1 model
Mutations in DJ-1 (PARK7) are the least common (1–2%) in autosomal recessive PD.31 DJ-1 is an antioxidant stress sensor167,168 that forms dimers to execute its normal function. In disease conditions, the defective protein is rapidly degraded because of its inability to form dimers.169,170 While mutations in DJ-1 were first described in 2002, it is only recently that a mouse model with clear dopaminergic neuron loss was described.171 These mice display mild motor behavior deficits upon aging.171 In flies, loss of DJ-1 results in reduced climbing ability and, in aged flies, reduced mitochondrial function.172 As these symptoms are similar to those observed in parkin and Pink1 mutant flies, it was also tested if overexpression of DJ-1 could overcome the observed defects in these mutant flies. Only Pink1-dependent mitochondrial defects could be rescued, while parkin mutant flies did not show improved mitochondrial function,172 suggesting DJ-1 function may be related to ETC function and acts downstream or in a parallel pathway of Pink1.

How does this work lead to drugs?
Several PD animal models show mitochondrial defects that, at the macroscopic level, appear rather similar. However, at the molecular and pathway level they may be affecting different aspects of mitochondrial function and/or quality control. It will therefore be important to further characterize the models and to assess what strategies are “above” the molecular differences and what strategies are specific to each of the models, the latter strategy probably being the more targeted approach that may result in less severe side effects. In the case of MPTP or rotenone intoxication, PINK1, and DJ-1, it seems that targeting ETC function may be beneficial (Fig. 4). Ret, the signaling receptor of glial cell–line derived neurotrophic factor, is a complex I regulator, as it can rescue both MPTP-induced PD models as well as PINK1-related PD symptoms by increasing complex I activity.164,173,174 Also, the expression of the yeast complex I improves complex I activity, overcoming mitochondrial defects in both loss of Pink1 and complex I.144 While complex I is defective in PINK1 mutants, there are several entry points for electrons into the ETC and stimulation of electron transport in downstream ETC complexes may also improve mitochondrial function. Stimulation of the ETC by administering vitamin K2 that functions as an electron carrier,145 or near-infrared light stimulation that increases complex IV activity, can improve mitochondrial function.166 These ETC stimulations have also been shown to improve mitochondrial defects in rotenone-induced toxicity.145,166 Thus, pharmacological stimulation of the ETC is an interesting potential therapeutic approach that has proven its effectiveness in some of the PD models. Additional translational work is now needed.

As previously mentioned, Parkin is an E3 ligase involved in mitochondrial clearance, but it may also harbor additional targets, some of which are located at the synapse. Pathogenic parkin mutations are partial (or complete) loss-of-function mutations that would therefore lead to reduced target ubiquitination. Hence, inhibition of deubiquitinases that deubiquitinate Parkin targets could be used to bring back balance to the ubiquitination status of the Parkin targets, even without knowing the identity of all of the targets, thereby re-establishing the regulation of their break-down. The recent identification of USP15 and USP30 may facilitate the identification of such inhibitory compounds. Additionally, with the current knowledge on cytosolic targets of Parkin, including PARIS and AIMP2, new pharmacological approaches can be proposed. The discovery of upstream elements in the Parkin ubiquitination cascade is of great relevance. A good example is c-Abl, which phosphorylates Parkin and inhibits its function, leading to the accumulation of Parkin targets.175 In vivo, c-Abl inhibition restores the levels of the Parkin substrate PARIS, resulting in prevention of dopamine neuron loss and motor dysfunction after MPTP treatment.176,177 However, AIMP2 levels were not restored upon c-Abl inhibition, suggesting that the protective effect may, in part, be Parkin independent.176 An increased knowledge on Parkin targets and upstream regulators is essential to a better and specific modulation of Parkin activity that can be used as a therapy and prevent PD progression.

Newly discovered genes and pathways
While early identified genes have been the main focus of PD research, newly discovered genes are beginning to give rise to new animal models and
Figure 4. Mitochondrial dysfunction and therapies in Parkinson’s disease. (Top) When mitochondrial membrane potential is disrupted, PINK1 remains uncleaved at the mitochondrial membrane, sending a signal for Parkin to be recruited to the mitochondria. At the mitochondria, Parkin ubiquitinates mitochondrial proteins in preparation for mitochondrial degradation, also termed mitophagy. Deubiquitinases can block the ubiquitination of mitochondrial proteins, preventing mitophagy from occurring. Upon loss of Parkin, deubiquitination results in an improvement of Parkin-related phenotypes and thus could be a valid novel therapeutic strategy. (Bottom) PINK1 deficiency, rotenone, and MPTP all result in reduced activity of complex I of the ETC, resulting in PD. By increasing the efficiency of the ETC via electron transport molecules, such as ubiquinone or vitamin K2, or via stimulation via infrared light, symptoms can be reduced in these mitochondrial forms of PD. A similar strategy could hold potential as a novel therapeutic for PD.

additional pathways that appear defective in some PD cases. Owing to their recent classification as PD causative genes, these animal models are not yet fully characterized. Here, we summarize the phenotypes observed in these newly developed tools for studying PD.

**UCH-L1**
Classically, ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) was described as a deubiquitinating enzyme that removes ubiquitin from a selection of target proteins. Recently, ubiquitin ligase activity was also described for this abundant enzyme in the brain (1–2% of the total protein). An autosomal dominant point mutation in UCH-L1 (I93M) disrupts the hydrolytic activity of the enzyme and is linked to PD. However, studies suggest that UCH-L1 mutations cannot completely explain the onset of PD. Mutations in this protein do not lead to fully penetrant PD phenotypes in mice. Additionally, mice with mutations on UCH-L1 do not present all of the characteristic defects of PD. In fact, UCH-L1 mutant mice present synaptic function defects with progressive loss of presynaptic terminals. Mutations in UCH-L1 impair autophagy and could increase the propensity to α-syn accumulation. Indeed, α-syn–associated neurotoxicity is enhanced by mutations in UCH-L1. Since these two proteins physically interact, UCH-L1 is also able to modify α-syn levels and interactions with other proteins. In conclusion, although UCH-L1 can be considered a risk factor for PD, the causative link between mutations in this gene and PD is not yet entirely clear.

**ATP13A2**
The gene Atp13a2 encodes a lysosomal P-type ATPase; mutations in this gene lead to several
lysosomal alterations and increase the risk for early-onset PD.\textsuperscript{188} Mice lacking this protein show subtle PD phenotypes upon aging (20–29 months old), including decreased locomotion and protein accumulation; however, no dopaminergic neuron loss was observed.\textsuperscript{189} Since \textit{Atp13a2} mutations cause early-onset PD and phenotypes in mice are only revealed upon aging, new animal models are needed to study the relevance of this gene in PD.

\textit{Atp13a2} has a neuroprotective function in aged brains and its mRNA levels are increased in the brains of late-onset PD.\textsuperscript{190} Overexpression of the yeast ortholog of \textit{Atp13a2} suppressed \(\alpha\)-syn toxicity, thus suggesting that this gene is required for responses to a stressful stimulus that is implicated in PD.\textsuperscript{191} Thus, combining \textit{Atp13a2} mutations with other factors that promote PD onset could lead to interesting PD models that would contribute to the general understanding of PD mechanisms and to novel therapeutic approaches.

\textbf{HtrA2/Omi}

\textit{HtrA2/Omi} encodes a serine protease localized to mitochondrial intermembrane space.\textsuperscript{192} PD-associated autosomal dominant mutations in \textit{HtrA2/Omi} lead to altered mitochondrial morphology and consequently to mitochondrial dysfunction.\textsuperscript{193} A141S and G399S mutations lead to deficient phosphorylation of HtrA2/Omi in a PINK1-dependent manner.\textsuperscript{193,194} Additionally, HtrA2 phosphorylation is decreased in the brains of PD patients carrying mutations in PINK1.\textsuperscript{194}

Mice with a deletion of \textit{HtrA2/Omi} have reduced body weight, a progressive loss of neurons in the striatum, and motor problems.\textsuperscript{195} The deletion of \textit{HtrA2/Omi} in mice triggers the translation of nuclear genes responsible for activation of the stress response, accumulation of unfolded proteins in the mitochondria, defective mitochondrial respiration, and enhanced production of reactive oxygen species.\textsuperscript{196} Deletion of this gene in \textit{D. melanogaster} results in motor deficits without any of the other PD-related phenotypes.\textsuperscript{197} Nonetheless, further studies are still required to understand the relevance of \textit{HtrA2/Omi} in PD.

\textbf{Synaptojanin}

Recently, mutations in gene encoding synaptojanin (SYNJ) have been identified in autosomal recessive cases of PD.\textsuperscript{198,199} Synaptojanin is a well-documented protein involved in endocytosis.\textsuperscript{200–202} Interestingly, recessive mutations in \textit{SYNJ} in model organisms cause defects in synaptic transmission as a result of defective synaptic vesicle endocytosis, somewhat similar but much more severe in comparison to the dominant mutations in LRRK2 and \(\alpha\)-syn. Several animal models have been created to study the function of \textit{SYNJ} in mice, flies, and \textit{Caenorhabditis elegans},\textsuperscript{200–202} however, since this gene was only recently connected to PD, these animals were never tested for phenotypes that can be related to PD. Although researchers are only now focusing on this genetic form of PD, mutations in this gene could be of relevance to the study of synaptic impairments in PD.

\textbf{\(\beta\)-Glucocerebrosidase}

Mutations in \(\beta\)-glucocerebrosidase (\textit{GBA}) are associated with PD.\textsuperscript{203} Although mutations in this gene are mostly studied because of their relevance in Gaucher’s disease, the most common lysosomal storage disorder, they are also linked to increased risk for PD.\textsuperscript{204} PD patients carrying \textit{GBA} mutations have widespread and abundant \(\alpha\)-syn pathology,\textsuperscript{203} decreased release of dopamine, and are responsive to levodopa treatment.\textsuperscript{205}

Consistent with a possible relation between \textit{GBA} mutations and \(\alpha\)-syn aggregation, pharmacological inhibition of GBA was recently shown to increase \(\alpha\)-syn accumulation in mice.\textsuperscript{206} However, owing to the recent discovery of \textit{GBA} as a causative gene for PD, the molecular mechanisms underlying the relation between GBA activity and \(\alpha\)-syn accumulation are unknown.

\textbf{Parkinson’s disease heterogeneity implies a patient-specific treatment}

In general, rodent models of PD are able to reproduce well the effects of dopaminergic medications known to be effective in PD patients in the reestablishment of motor performance.\textsuperscript{207} The most used therapy in PD patients, \textit{l}-DOPA, but also dopamine receptor agonists and monoamine oxidase inhibitors, can alleviate symptoms in animal models.\textsuperscript{208} However, the effectiveness of these therapeutic approaches is limited in time with the consequent loss of effect. Currently available PD treatments merely alleviate the symptoms of the disease and are inadequate to tackle the effects that lead to neuronal dysfunction and death in PD. Given the relevance of the aging process in PD and the
increased life expectancy of the world population, the incidence of this disease is growing dramatically. Therefore, new approaches to treat this disease are urgently needed not only to cease but mostly to revert PD as soon as it is diagnosed, or ideally, before its onset. Research on animal models suggests the existence of separate pathways, including those affecting mitochondria and those affecting vesicle trafficking, that eventually converge to cause similar symptoms. While several of the dominant mutations are associated with synaptic deficits, recessive forms of PD usually lead to mitochondrial dysfunction. Findings in animal models can be used to promote clinical trials and these studies should clarify which treatments will affect each of the pathways involved in PD. Indeed, most of the clinical trials for new drugs and therapeutic approaches failed, possibly because patients were not stratified sufficiently according to PD subtype. The existence of these initial separate pathways allows specific targeting of the affected pathway and highlights the need for stratification of patients in order to select the appropriate treatment. Indeed, patients may benefit much more from a specific therapeutic approach rather than general symptomatic treatments.

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Conflicts of interest

The authors declare no conflicts of interest.

References


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