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**REVIEW ARTICLE**

# Parkinson's disease: what the model systems have taught us so far

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**Abstract.** Parkinson's disease (PD) is a debilitating neurodegenerative disorder, for which people above the age of 60 show an increased risk. Although there has been great advancement in understanding the disease-related abnormalities in brain circuitry and development of symptomatic treatments, a cure for PD remains elusive. The discovery of PD associated gene mutations and environmental toxins have yielded animal models of the disease. These models could recapitulate several key aspects of PD, and provide more insights into the disease pathogenesis. They have also revealed novel aspects of the disease mechanism including noncell autonomous events and spreading of pathogenic protein species across the brain. Nevertheless, none of these models so far can comprehensively represent all aspects of the human disease. While the field is still searching for the perfect model system, recent developments in stem cell biology have provided a new dimension to modelling PD, especially doing it in a patient-specific manner. In the current review, we attempt to summarize the key findings in the areas discussed above, and highlight how the core PD pathology distinguishes itself from other neurodegenerative disorders while also resembling them in many aspects.

**Keywords.** Parkinson's disease; neurodegenerative disease; cell reprogramming; stem cells.

## Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease, and is characterized by progressive decline of motor and cognitive functions in affected individuals. At the time of seeking medical help, most of these patients would have already incurred a loss of over 70% of the dopaminergic (DA) neurons of the substantia nigra pars compacta (SNpc). Clinically they manifest motor dysfunctions including increased resistance to passive limb movement (rigidity), slowness of movement (bradykinesia), reduction in movement amplitude (hypokinesia), absence of normal unconscious movements (akinesia), paucity of normal facial expression (hypomimia) and decreased voice volume (hypophrenia) (Dauer and Przedborski 2003; Ellens and Leventhal 2013). The most common initial therapy for PD is the administration of levodopa (L-Dopa), a prodrug to dopamine. While patients often show good response initially, with the passage of time most of them experience alternative phases of good (on-state) and poor (off-state) fluctuations in response to medication, besides developing other motor complications including dyskinesia. Other drugs like monoamine oxidase type B inhibitors, catechol-O-methyltransferase inhibitors, the NMDA receptor antagonist amantadine and dopamine receptor agonists are used to manage these fluctuations (Jankovic and Stacy 2007). Deep brain electrical stimulation is also used to control the motor symptoms in some patients who do not respond well to medications (Hammond *et al.* 2007). However, two centuries since PD was first described, a therapy for modifying or halting the progression of the disease remains elusive. In the current review, we focus on the different model systems available to study PD, and what they have taught us so far in understanding the pathology of the disease. We conclude with a discussion on how the recent developments in stem cell biology could potentially transform the field and provide us with new hope for developing a curative therapy for the disease.

Swagata Ghatak and Dorit Trudler contributed equally to this work.

## PD: a disease of circuit dysfunction

The motor deficits seen in PD patients can be traced back to defective neuronal circuits in the motor centres of their brains (Meredith and Wouterlood 1990; Lapper and Bolam 1992; Sidibe and Smith 1996). Parkinsonism is considered to result primarily from abnormalities of basal ganglia function. The basal ganglia include the neostriatum, the external and internal pallidal segments, the subthalamic nucleus, and the substantia nigra, including pars reticulata (SNpr) and pars compacta (SNpc). They participate in anatomically and functionally segregated loops (motor, associative and limbic loops) involving specific thalamic and cortical areas (Alexander *et al.* 1986, 1990; Middleton and Strick 2000; Kelly and Strick 2004).

In a functional motor circuit, the striatum and subthalamic nucleus receive glutamatergic afferents from specific areas of the cerebral cortex or thalamus, and transfer the information to the basal ganglia and SNpc. The SNpc in turn sends prominent DA projections to the striatum where they terminate predominately at the dendritic spines of the striatal medium spiny neurons (MSNs). MSN spines also receive corticostriatal terminations. Anatomically, the DA inputs are in a position to regulate the corticostriatal transmission. The projections between the striatum and SNpc are divided into two separate pathways, a direct monosynaptic connection, and an indirect connection. Output from the SNpc goes largely to the thalamus, which in turn projects back to the cerebral cortex. Dopamine release from the nigrostriatal projection facilitates corticostriatal synaptic transmission onto direct pathway-MSNs, and inhibits transmission along indirect pathway-MSNs thereby enabling movement (DeLong and Wichmann 2007).

In PD, chronic dopamine loss leads to changes in the firing rates of basal ganglia neurons that follow the rate model predictions (figure 1). According to the rate model, striatal dopamine enhances direct MSN firing, inhibits indirect MSN firing and thereby suppresses basal ganglia output, releasing thalamocortical circuits from tonic inhibition. Dopamine loss causes dysregulation of corticostriatal transmission rather than affecting the spontaneous activity of MSNs directly. Loss of dopamine D2 receptor activation decreases inhibition of indirect-pathway MSNs causing increased corticostriatal transmission. This in turn increases inhibition of external pallidal segment activity and disinhibits the subthalamic nucleus, eventually leading to excessive activity in the substantia nigra. In addition, the loss of dopamine decreases activation of striatal dopamine D1 receptors, which may disinhibit the internal pallidal segment and substantia nigra along the direct pathway. The resulting increase in activity of these neurons can lead to greater inhibition of neurons in the thalamus and brainstem (Albin *et al.* 1989; DeLong 1990) leading to the motor symptoms seen in patients.

During the disease progression, the dopaminergic SNpc neurons and their projections to the striatum degenerate slowly (DeLong 1990). However, projections to the putamen, which constitute the motor loop, degenerate before projections to limbic or associative areas of the striatum, causing the motor symptoms of PD to develop before the nonmotor signs (Galvan and Wichmann 2008; Aarsland *et al.* 2017). Recognizable PD symptoms appear only after substantial neurodegeneration in the substantia nigra because of the extraordinary compensatory mechanisms within the dopaminergic system (Bernheimer *et al.* 1973).

The chronic functional changes that occur during Parkinsonism include biochemical and anatomical changes in the function of GABAergic and glutamatergic pathways in the basal ganglia. Changes in GABA levels and GABA receptor binding or expression has been implicated in the pathophysiology, but involvement of ionotropic as well as metabotropic glutamate receptors in PD is still not clear (Chadha *et al.* 2000; Dunah *et al.* 2000; Betarbet *et al.* 2004; Katz *et al.* 2005; Samadi *et al.* 2008; Ellens and Leventhal 2013). The downregulation of NMDA and AMPA receptors in the basal ganglia output nuclei in dopamine-depleted animals and PD patients could be a compensatory mechanism reflecting enhanced activity in the glutamatergic connections of the subthalamic nucleus (Dunah *et al.* 2000; Betarbet *et al.* 2004).

In patients with PD, there is an increase in burst firing in the basal ganglia (Hutchison *et al.* 1994; Magnin *et al.* 2000). Bursting activity in the subthalamic nucleus develops during dopamine depletion and is associated with changes in firing rates and metabolic markers (Breit *et al.* 2007). Similar observations have been made in dopamine-depleted monkeys that show distinct changes in burst firing in the pallidal segments and subthalamic nucleus (Soares *et al.* 2004; Wichmann and Soares 2006; Ellens and Leventhal 2013). The proportions of spikes within bursts, the time for which the neurons are bursting, and the length of individual bursts are all increased (Soares *et al.* 2004). Although it is likely that excessive burst discharges alter basal ganglia-thalamocortical circuitry, it remains unknown if bursting has proparkinsonian effects, because antiparkinsonian dopaminergic treatments do not typically decrease burst discharges in the basal ganglia of dopamine-depleted animals or patients.

Another prominent abnormality in the neuronal activity of basal ganglia in parkinsonian animals and patients is the appearance of abnormal oscillatory activity, both at the single-cell level and in neural networks (Levy *et al.* 2002a,b; Gatev *et al.* 2006; Rivlin-Etzion *et al.* 2006).

## Pathological hallmarks

The aetiology of PD remains poorly understood. While environmental factors, gene mutations and susceptibility

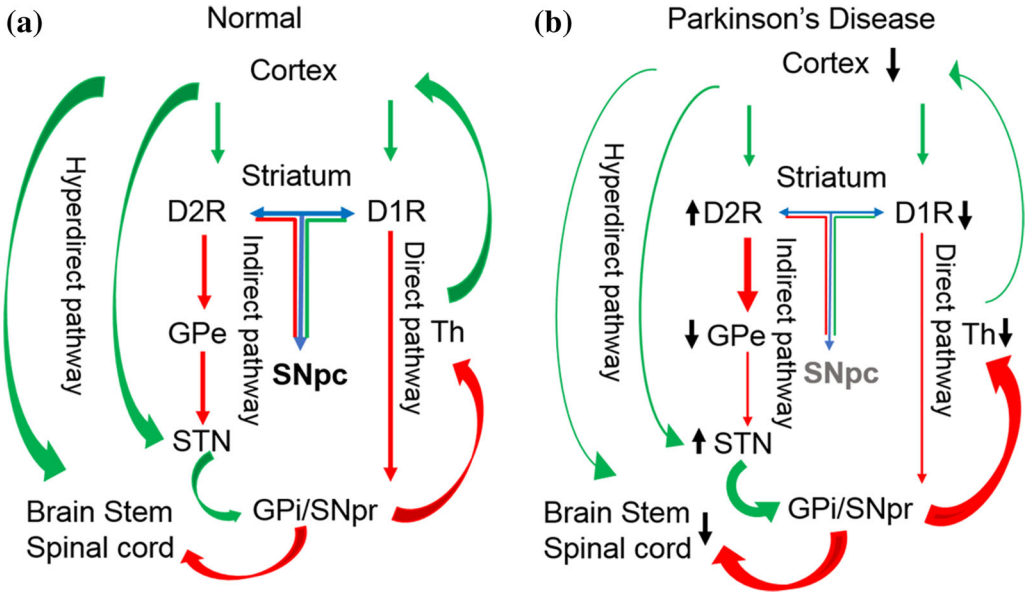


**Figure 1.** Schematic representation of the rate model of PD. **(a)** Normal cortico-thalamic circuit. **(b)** Changes in the cortico-thalamic circuit in PD. Green, red and blue arrows represent glutamatergic/excitatory, GABAergic/inhibitory and dopaminergic neurons, respectively. The weight of the arrow represents the strength of the input (thick, more; thin, less). D1R, D2R, dopamine D1 and D2 receptors; GPe and GPi, external and internal segments of the globus pallidus; SNpc (black, normal; grey, degenerated) and SNpr, substantia nigra pars compacta and reticulata; STN, subthalamic nucleus; Str, striatum; Th, thalamus. The black arrows beside the brain regions represents total activity of the area (upward, increased activity; downward, decreased activity). (Adapted from Smith *et al.* (2012); figure 1).

loci (from genomewide association studies) are implicated, there is little information as yet regarding what triggers the disease pathology or drives its progression. The pathological hallmarks of PD are the loss of the dopaminergic neurons of the SNpc and the existence of intraneuronal cytoplasmic proteinaceous spherical inclusions called Lewy bodies and Lewy neurites. In the presymptomatic stages of the disease, the inclusion bodies are confined to the medulla oblongata and the olfactory bulb. In early stages of the disease, the substantia nigra and other nuclei of the midbrain and forebrain become affected. It is this stage when patients start developing clinical symptoms. At a later stage, degeneration spreads to the neocortex marking the final phase of the disease.

The role of Lewy bodies in the pathologic process underlying PD is unknown. However, understanding the nature and mechanism of Lewy body formation has been triggered by the finding that the protein α-synuclein (encoded by the *SNCA* gene) is a major component of these structures (Spillantini *et al.* 1997; Takahashi and Wakabayashi 2001). Because of its tendency to self-aggregate (Conway *et al.* 1998), it has been hypothesized that α-synuclein may be responsible for the formation and deposition of insoluble pathologic inclusions which may ultimately play a role in neurodegeneration (Takahashi and Wakabayashi 2001). Toxic insults could both modify the structure of α-synuclein and interfere with the ubiquitin-proteasomal pathway, thus promoting α-synuclein aggregation and impairing the process of degradation of the abnormal protein. Studies have shown direct evidence of interactions between α-synuclein and environmental agents such that incubations of α-synuclein with certain pesticides dramatically accelerated the rate of α-synuclein fibrillation *in vitro* (Uversky 2003). Given the association of toxins and gene mutations with PD, a number of animal models have been generated utilizing these factors to mimic the disease pathology.

Spread of α-synuclein and Lewy bodies are associated with the nonmotor symptoms of PD caused by damage to the cerebrocortex and other brain areas (Hurtig *et al.* 2000). The most common nonmotor symptom in PD is dementia, with a prevalence of about 80% in those who were diagnosed with PD 15–20 years ago (Biundo *et al.* 2016). Patients initially show mild cognitive impairment (MCI), which can subsequently develop into frank dementia. These symptoms can also signal other neurodegenerative diseases such as Alzheimer's disease (AD). In fact, 15–20% of the PD cases with dementia will also have coexiting AD. A subset of PD patients also exhibit additional cognitive impairments, including deficits in language and executive function. Additional psychiatric symptoms include hallucinations and delusions, particularly in patients with Lewy body dementia (LBD) (Diederich *et al.* 2009).

Cognitive deficits may arise from the dysfunction of both motor and nonmotor circuits. Dopamine depletion in the nigro-striatal pathway and in cortical circuits

may result in deficits in working memory, memory recall and psychomotor speed (Goldman *et al.* 2014; Biundo *et al.* 2016). Since PD treatments focus on increasing dopamine levels, these treatments can also produce an overdose of dopamine in the nondamaged portion of the circuits. This can cause detrimental effects on orbito-frontal cortex-related executive functions (Biundo *et al.* 2016). Dopaminergic therapy can also cause compulsive gambling, confusion, delirium, and psychotic-like behaviour, which are often treated with antipsychotic drugs. Recently, pimavanserin (Nuplazid) received a new drug approval from the ford and drug administration (FDA) to treat hallucinations and delusions associated with psychosis experienced by some people with PD/LBD (Combs and Cox 2017). Since pimavanserin acts as a serotonin 2A receptor inverse agonist, and not on dopaminergic pathways, it prevents cognitive symptoms without interfering with the conventional treatment of motor symptoms (Meltzer *et al.* 2010).

Gastrointestinal (GI) symptoms are frequently reported by patients at all stages of PD (Pfeiffer *et al.* 2003), and several recent studies have proposed a potential role for the gut in PD pathogenesis (Clairembault *et al.* 2015; Klingelhoefer and Reichmann 2015). An early nonmotor symptom of PD is chronic constipation. The observation of lower dopamine levels in the GI tract in severely constipated PD patients suggests a role for a damaged enteric dopaminergic system in GI dysfunction (Singaram *et al.* 1995). The enteric nervous system (ENS) is a major part of the autonomic nervous system that forms a network of neurons innervating the GI wall. These neurons play an important role in the bidirectional communication between the central nervous system (CNS) and the GI tract, often described as the gut–brain axis (Cryan and Dinan 2012; Perez-Pardo *et al.* 2017b), and regulate normal gut motility through production of dopamine (Li *et al.* 2004). An altered dopaminergic ENS circuitry may result in decreased gut contraction and slower GI transit in PD patients (Sakakibara *et al.* 2008). Consistent with the observations in humans, others have reported progressive deficits in gut motility, increased α-synuclein expression in the colon and compromised intestinal and colonic barrier integrity in PD mouse models (Chesselet *et al.* 2012; Perez-Pardo *et al.* 2017a).

As per the Braak staging system of PD, Lewy body pathology and neurodegeneration initiates in the olfactory bulb and the ENS before spreading to the CNS (Braak *et al.* 2006). Although a few subsequent studies have challenged this idea (Attems and Jellinger 2008; Kalaitzakis *et al.* 2008), others reported Lewy inclusions in the oesophagus and α-synuclein staining in GI tissues of patients at preclinical stages of the disease (Hilton *et al.* 2014), thus supporting Braak's hypothesis. Interestingly, removal of the entire vagus nerve offered protection against PD in patients (Svensson *et al.* 2015). Consistent with this finding, human α-synuclein introduced into the intestinal wall of rats could migrate up the vagus nerve to the dorsal motor nucleus (DMV) of the brain stem (Houser and Tansey 2017).

Recent evidence suggests that gut microbiota might influence gut–brain interactions (Grenham *et al.* 2011; Borre *et al.* 2014). PD patients show an increased intestinal permeability to bacterial products like LPS that leads to inflammation and oxidative stress in the GI tract. GI tract inflammation has been suggested as a trigger for α-synuclein accumulation in the ENS (Glass *et al.* 2010; Forsyth *et al.* 2011). Additionally, gut-derived LPS can facilitate neuroinflammation and injury in the SNc through disruption of the blood brain barrier (Banks *et al.* 2008; Banks and Erickson 2010). Studies have shown specific microbial metabolites like short chain fatty acids to be sufficient to promote α-synuclein mediated neuroinflammation and motor deficits observed in PD (Sampson *et al.* 2016). Interestingly, fecal microbes from PD patients transplanted in mice show significant motor function impairment compared to microbiota from healthy controls, suggesting a critical role of gut microbes in PD pathogenesis (Sampson *et al.* 2016).

Although the above studies point to an association of GI tract phenotypes and PD, mechanisms that directly connect these phenotypes to the core PD-specific pathology remain unclear.

## Environmental toxins and toxin-based models

A common feature of all toxin-induced models is their ability to produce oxidative stress and to cause cell death in dopaminergic neuronal populations that reflect what is seen in human PD patients (Blesa *et al.* 2012). The toxins used to induce PD symptoms include 6-hydroxydopamine (6-OHDA), 1-methyl-1,2,3,6-tetrahydropyridine (MPTP), paraquat (*N,N*-dimethyl-4-4-4-bypiridinium, PQ), and rotenone.

### *6-Hydroxydopamine (6-OHDA)*

The neurotoxin 6-OHDA is structurally similar to dopamine and has a high affinity for its plasma membrane transporter (Breese and Traylor 1971). Once inside a neuron, 6-OHDA is readily oxidized, producing hydrogen peroxide and paraquinone, both of which are highly toxic (Saner and Thoenen 1971). This toxin does not cross the blood-brain-barrier (BBB) but, when administered directly to the brain, specifically kills dopaminergic neurons and their terminals (Javoy *et al.* 1976). The degree of loss of dopaminergic neurons and their striatal terminals depends on the location and dose of the toxin. In the rodent 6-OHDA model, dopamine depletion, nigral dopamine cell loss, and neurobehavioural deficits are observed

(Blesa *et al.* 2012). However, this toxin does not produce extranigral pathology or Lewy body-like inclusions (Lane and Dunnett 2008).

#### *Mitochondrial complex-I inhibitors*

Defects in complex I may be central to the pathogenesis of PD (Dawson and Dawson 2003). In rodent and primate models, several complex I inhibitors such as MPTP (Smith *et al.* 1997) and rotenone (Betarbet *et al.* 2000) cause selective dopaminergic cell death and lead to the formation of inclusions similar to Lewy bodies, the pathologic hallmark of PD (Betarbet *et al.* 2000; Vila and Przedborski 2003).

***MPTP***: Is one example of an exogenous toxin that causes people to develop clinical and pathological features of PD (Langston *et al.* 1999). It is highly lipophilic and after systemic administration rapidly crosses the BBB. Once in the brain, MPTP enters astrocytes and is metabolized into $MPP^+$, its active metabolite, by monoamine oxidase B (MAO-B). $MPP^+$ is internalized by neurons through the dopamine transporter and can be stored in vesicles via uptake by the vesicular monoamine transporter 2 (VMAT2) (Javitch *et al.* 1985). Once inside the neuron, $MPP^+$ is able to inhibit mitochondrial complex I, resulting in the release of ROS and reduced ATP production (Burke *et al.* 2008).

***Rotenone***: A compound used as an insecticide, is also highly lipophilic and readily crosses the BBB. Rotenone inhibits mitochondrial complex I by binding to the acceptor and causing an increase in the reduction state of the complex, thus leading to the leakage of electrons that combine with oxygen to form superoxide (Nicholls 2008). Complex I inhibition by rotenone leads to initiation of several deleterious effects that include generation of ROS that result in oxidative stress, reduction of ATP levels, and apoptotic or necrotic cell death (Subramaniam and Chesselet 2013). Intravenous administration of rotanone in rodents causes damage to nigrostriatal DA neurons and is accompanied by α-Syn aggregation, Lewy body formation, oxidative stress, and gastrointestinal problems (Cannon *et al.* 2009). However, the role of rotenone in causing PD like symptoms in humans is contentious as this compound is highly unstable in solution (Bove *et al.* 2005).

***Paraquat***: Is a widely used herbicide that structurally resembles $MPP^+$. PQ exerts its deleterious effects through oxidative stress mediated by redox cycling, which generates ROS (Przedborski and Ischiropoulos 2005). PQ can induce increases in α-Syn protein levels that may result in Lewy body-like structures in dopaminergic neurons in the SNpc (Manning-Bog *et al.* 2002). Epidemiological reports suggest that use of pesticide increases the risk of PD, but there have been only a few cases of PD directly linked to PQ toxicity in humans (Berry *et al.* 2010).

### Mediators of neuroinflammation

In addition to the toxin models, other animal models of PD, which are characterized by increased inflammation leading to oxidative stress and neurodegeneration have been reported. Lipopolysaccharide (LPS), an endotoxin from gram-negative bacteria, is a potent inducer of inflammation that has diverse effects on microglia and astrocytes (Benveniste 1992). Inflammatory activation by intracerebral LPS administration into the cortex, hippocampus, striatum, or substantia nigra of mice and rats enhances the death of only dopaminergic neurons (Castano *et al.* 1998; Herrera *et al.* 2000), which is in accord with the inflammatory response reported in PD. Further, Qin *et al.* (2007) have reported that a single intraperitoneal injection of LPS in mice can initiate TNFα production in the brain that continues for months even after the systemic source of TNFα has been withdrawn. The delayed and progressive loss of DA neurons in the substantia nigra (over months) of these recipient mice, mimics human PD.

Most toxin-based models of PD discussed above take advantage of the ability of the toxins to damage the nigrostriatal dopaminergic system, resulting in diminished striatal dopamine levels and ensuing parkinsonism in animals. These models can be generated quickly on-demand and they produce certain PD phenotypes that can be used to examine new treatment approaches targeting striatal dopamine replacement or PD related symptoms. However, these acute models seldom recapitulate normal disease aetiology, i.e. slow progression and wide-spread neurodegeneartion, associated with the human disease. These shortcomings severely limit their utility in testing disease modifying therapies for PD. To address these limitations, gene-based models have been developed and are constantly being refined.

### Gene mutations and gene-based models

Although the cause of sporadic PD is unclear, several gene mutations and risk alleles associated with single-nucleotide polymorphisms (SNP) have been linked to the disease pathology (Wood-Kaczmar *et al.* 2006; Xiromerisiou *et al.* 2010; Nalls *et al.* 2014; Chang *et al.* 2017; Obeso *et al.* 2017) (table 1). For instance, 5–10% of those who exhibit PD clinical symptoms are found to carry mutations in one of the known 17 genes that cause monogenic forms of the disease (Carr *et al.* 2003). These mutations can cause either loss or gain of function in their respective proteins and can result in autosomal recessive or autosomal dominant PD (Wood-Kaczmar *et al.* 2006; Xiromerisiou *et al.* 2010), as discussed below.

**Table 1.** Genetic risk variants in PD. Summary of identified SNP from GWAS, and the candidate genes for these SNPs.

| SNP | Nearest/candidate gene | Odds ratio | Effect allele/ alternate allele | Reference |
|---|---|---|---|---|
| rs10797576 | *SIPA1L2* | 1.131 | T/C | Nalls *et al.* (2014); Obeso *et al.* (2017) |
| rs10906923 | *FAM171A1* | 0.91–0.96 | C/A | Chang *et al.* (2017) |
| rs11060180 | *CCDC62* | 1.105 | A/G | Nalls *et al.* (2014); Obeso *et al.* (2017) |
| rs11158026 | *GCH1* | 0.904 | T/C | Nalls *et al.* (2014) |
| rs11158026 | *GCH1* | 1.106 | C/T | Obeso *et al.* (2017) |
| rs11343 | *COQ7* | 1.05–1.10 | T/G | Chang *et al.* (2017) |
| rs114138760 | *PMVK* (*GBA*) | 1.574 | C/G | Obeso *et al.* (2017) |
| rs115185635 | *KRT8P25-APOOP2* | 1.142 | C/G | Nalls *et al.* (2014) |
| rs11724635 | *BST1* | 1.126 | A/C | Nalls *et al.* (2014); Obeso *et al.* 2017 |
| rs117896735 | *INPP5F* | 1.624 | A/G | Nalls *et al.* (2014); Obeso *et al.* (2017) |
| rs12456492 | *RIT2* | 0.904 | A/G | Nalls *et al.* (2014) |
| rs12456492 | *RIT2* | 1.106 | G/A | Obeso *et al.* (2017) |
| rs12497850 | *NCKIPSD, CDC71* | 0.91–0.96 | G/T | Chang *et al.* (2017) |
| rs12637471 | *MCCC1* | 0.842 | A/G | Nalls *et al.* (2014) |
| rs12637471 | *MCCC1* | 1.188 | G/A | Obeso *et al.* (2017) |
| rs13201101 | *C6orf10* | 1.192 | T/C | Obeso *et al.* (2017) |
| rs13294100 | *SH3GL2* | 0.89–0.94 | T/G | Chang *et al.* (2017) |
| rs14235 | *BCKDK-STX1B* | 1.103 | A/G | Nalls *et al.* (2014); Obeso *et al.* (2017) |
| rs143918452 | *ALAS1*, *TLR9*, *DNAH1*, *BAP1*, *PHF7*, *NISCH*, *STAB1*, *ITIH3*, *ITIH4* | 0.60–0.78 | G/A | Chang *et al.* (2017) |
| rs1474055 | *STK39* | 1.214 | T/C | Nalls *et al.* (2014); Obeso *et al.* (2017) |
| rs1555399a | *TMEM229B* | 0.897 | A/T | Nalls *et al.* (2014) |
| rs17649553 | *MAPT* | 0.769 | T/C | Nalls *et al.* (2014) |
| rs17649553 | *MAPT* | 1.300 | G/A | Obeso *et al.* (2017) |
| rs199347 | *GPNMB* | 1.110 | A/G | Nalls *et al.* (2014); Obeso *et al.* (2017) |
| rs2280104 | *SORBS3, PDLIM2, C8orf58, BIN3* | 1.04–1.09 | T/C | Chang *et al.* (2017) |
| rs2414739 | *VPS13C* | 1.113 | A/G | Nalls *et al.* (2014); Obeso *et al.* (2017) |

**Table 1** (*contd*)

| SNP | Nearest/candidate gene | Odds ratio | Effect allele/ alternate allele | Reference |
|---|---|---|---|---|
| rs2694528 | *ELOVL7* | 1.11–1.20 | C/A | Chang *et al.* (2017) |
| rs2740594 | *CTSB* | 1.07–1.12 | A/G | Chang *et al.* (2017) |
| rs329648 | *MIR4697HG* | 1.105 | T/C | Nalls *et al.* (2014); Obeso *et al.* (2017) |
| rs34043159 | *IL1R2* | 1.06–1.10 | C/T | Chang *et al.* (2017) |
| rs34311866 | *TMEM175* (*GAK*) | 0.786–1.272 | G/A | Nalls *et al.* (2014); Obeso *et al.* (2017) |
| rs34884217 | *TMEM175* (*GAK*) | 1.247 | A/C | Obeso *et al.* (2017) |
| rs353116 | *SCN3A* | 0.92–0.96 | T/C | Chang *et al.* (2017) |
| rs356182 | *SNCA* | 0.76 | A/G | Nalls *et al.* (2014) |
| rs356182 | *SNCA* | 1.316 | C/T | Obeso *et al.* (2017) |
| rs35749011 | *SLC50A1* (*GBA*) | 1.824 | A/G | Nalls *et al.* (2014); Obeso *et al.* (2017) |
| rs3793947 | *DLG2* | 0.929 | A/G | Nalls *et al.* (2014) |
| rs4073221 | *SATB1* | 1.06–1.13 | G/T | Chang *et al.* (2017) |
| rs4653767 | *ITPKB* | 0.90–0.94 | C/T | Chang *et al.* (2017) |
| rs4784227 | *TOX3* | 1.06–1.12 | T/C | Chang *et al.* (2017) |
| rs601999 | *ATP6V0A1, PSMC3I, TUBG2* | NA | C/T | Chang *et al.* (2017) |
| rs62120679 | *SPPL2B* | 1.097 | T/C | Nalls *et al.* (2014) |
| rs6430538 | *ACMSD* | 0.875 | T/C | Nalls *et al.* (2014) |
| rs6430538 | *ACMSD* | 1.143 | C/T | Obeso *et al.* (2017) |
| rs6812193 | *FAM47E-SCARB2* | 0.907 | T/C | Nalls *et al.* (2014) |
| rs6812193 | *FAM47E* | 1.103 | C/T | Obeso *et al.* (2017) |
| rs7681154 | *SNCA* | 1.189 | C/A | Obeso *et al.* (2017) |
| rs76904798 | *LRRK2* | 1.155 | T/C | Nalls *et al.* (2014); Obeso *et al.* (2017) |
| rs78738012 | *ANK2, CAMK2D* | 1.09–1.17 | C/T | Chang *et al.* (2017) |
| rs8005172 | *GALC* | 1.05–1.10 | T/C | Chang *et al.* (2017) |
| rs8118008 | *DDRGK1* | 1.111 | A/G | Nalls *et al.* (2014); Obeso *et al.* (2017) |
| rs823118 | *RAB7L1-NUCKS1* | 1.122 | T/C | Nalls *et al.* (2014); Obeso *et al.* (2017) |
| rs9275326 | *HLA-DQB1* | 1.211 | C/T | Obeso *et al.* (2017) |
| rs9275326 | *HLA-DQB1* | 0.826 | T/C | Nalls *et al.* (2014) |
| rs9468199 | *ZNF184* | 1.08–1.14 | A/G | Chang *et al.* (2017) |

NA, not available.

#### α-Synuclein (SNCA) or PARK1

*SNCA* mutations that cause an autosomal dominant form of the disease are the first reported gene mutations associated with PD. The protein product of this gene, α-synuclein, is the major component of Lewy bodies (Spillantini *et al.* 1997; Xiromerisiou *et al.* 2010). α-Synuclein is predominately expressed in the brain, particularly in the neocortex, hippocampus, striatum, thalamus, and cerebellum, and mainly in presynaptic vesicles (Iwai *et al.* 1995). The distribution pattern of α-synuclein pathology within selectively vulnerable neuronal populations is considered to be closely linked to the clinical dysfunctions seen in PD, and has been an important factor in staging the disease progression based on Braak's hypothesis (Braak *et al.* 2004).

The normal function of α-synuclein is poorly understood, but it is implicated in the regulation of vesicle dynamics at the presynaptic membrane, and is important in learning and neuronal plasticity (Sidhu *et al.* 2004). Although the function of α-synuclein–lipid binding in the brain is unclear, it might be connected with lipid-mediated signalling, trafficking and metabolism (Wood-Kaczmar *et al.* 2006).

Elevated expression levels of α-synuclein in the brain can also lead to the development of PD (Singleton *et al.* 2003; Miller *et al.* 2004). This enhanced expression increases the deposition of soluble α-synuclein into insoluble aggregates (Miller *et al.* 2004). Recently, the toxic form of α-synuclein aggregation has received great attention, and different strains have been implicated in the pathology of PD. Fibrils have been identified as the toxic form resulting in neuronal death, whereas ribbons produce the histopathological symptoms observed in PD (Peelaerts *et al.* 2015).

Several mutations (A53T, A30P, E46K) as well as duplications and triplications of α-synuclein have been identified in familial cases of PD (Polymeropoulos *et al.* 1997; Kruger *et al.* 1998; Zarranz *et al.* 2004; Nishioka *et al.* 2006). Pathogenic mutations in α-synuclein (notably A53T) cause increased rates of protein self-assembly and fibrillization, and are considered gain-of-function mutations that follow an autosomal-dominant form of inheritance (Conway *et al.* 2000). Clinically, patients with mutated α-synuclein have a relatively young age of onset, rapid progression, and high prevalence of dementia, psychiatric, and autonomic disturbances (Xiromerisiou *et al.* 2010).

Mice that express human α-synuclein mutated at A53 to T under control of the prion promoter begin to develop motor dysfunction at eight months of age. The symptoms start with neglect of grooming accompanied by weight loss, which later progresses to movement impairments and freezing of the hind limbs. These mice also develop histopathological abnormalities including α-synuclein inclusions and neuronal death (Giasson *et al.* 2002).

Neurons that undergo stress can secrete α-synuclein (Luk and Lee 2014), which propagates across the brain as a prion like particle (Luk *et al.* 2012), and can subsequently activate microglia. In turn, microglia can enhance the local inflammatory environment surrounding α-synuclein-expressing neurons and may lead to abnormal handling of α-synuclein in neurons (Sanchez-Guajardo *et al.* 2013). The presence of abnormal α-synuclein expression in cells surrounding neuro-inflammatory lesions supports the idea that the neuron–microglia interactions that exacerbate the disease process can be initiated either by microglia or neurons (Lu *et al.* 2009).

#### Leucine-rich repeat kinase 2 (LRRK2) or PARK8

*LRRK2* encodes a 2527 amino acid cytoplasmic protein that consists of a leucine-rich repeat toward the amino terminus of the protein and a kinase domain toward the carboxyl terminus (Klein and Westenberger 2012). To date, there are more than 50 different missense and nonsense mutations reported in *LRRK2* (Nuytemans *et al.* 2010), and at least 16 of them are pathogenic with gain of function attributes, which cause an autosomal dominant form of disease. The *LRRK2* G2019S mutation has been extensively researched (West *et al.* 2005; Klein and Westenberger 2012) and is found in over 20% of PD patients of Ashkenazi Jewish descent and over 40% of PD patients with North African Berber Arabic ancestry. Notably, the clinical and pathological findings associated with this mutation are indistinguishable from sporadic PD. However, the pathogenic mechanism leading to PD caused by *LRRK2* mutations is still uncertain. Due to its many possible protein–protein interactions, it is plausible that changes in its interaction domains would influence LRRK2's relationship with other proteins (Venderova *et al.* 2009; Berger *et al.* 2010; Klein and Westenberger 2012).

*LRRK2* knockout mice and rats carry only subtle phenotypes (Hinkle *et al.* 2012; Daher *et al.* 2014; Xiong *et al.* 2017). Moreover, the G2019S and R1441C *LRRK2* mice develop normally (Xiong *et al.* 2017), suggesting that haploinsufficiency may not be the basis for the effect of these dominant mutations. Interestingly, mice overexpressing the G2019S mutation develop mild progressive and selective degeneration of dopaminergic neurons in the substantia nigra. These mice do not exhibit any motor behavioural deficits, in contrast to a *LRRK2* BAC transgenic mouse model (Xiong *et al.* 2017).

#### Parkin or PARK2

*Parkin* mutations are associated with familial PD as well as sporadic young onset PD. Parkin has E3 ubiquitin ligase activity (Shimura *et al.* 2000), and *Parkin* mutations in

patients result in the loss of E3 activity (Imai *et al.* 2000). While *parkin* knock-out mice do not exhibit any overt manifestations of PD, the *parkin*-Q311X-*DAT*-BAC transgenic mice display age-dependent degeneration of SNpc dopaminergic neurons and accompanying motor deficits relating to PD (Lu *et al.* 2009).

Parkin is abundantly expressed in microglia, and *parkin*-null mice have an increased number of microglia by immunocytochemical analysis (Casarejos *et al.* 2006). Additionally, parkin loss of function in microglia resulted in enhanced toxicity to dopaminergic neurons after rotenone treatment (Casarejos *et al.* 2006).

#### *PTEN induced putative kinase 1 (PINK1) or PARK6*

Mutations in *PINK1* account for 1–8% of the early onset autosomal recessive PD cases and are considered loss-of-function mutations (Klein *et al.* 2007; Xiromerisiou *et al.* 2010). Patients with mutations in this gene are characterized by slow progression and sustained response to L-Dopa (Albanese *et al.* 2005). There are also some indications that they have a higher prevalence of psychiatric disturbances (Ephraty *et al.* 2007). *PINK1* has an *N*-terminal mitochondrial targeting motif and a highly conserved kinase domain homologous to the serine/threonine kinases of the $Ca^{2+}$/calmodulin family (Gandhi *et al.* 2006).

PINK1 is expressed throughout the human brain, and it is found in all cell types with a punctate cytoplasmic staining pattern consistent with mitochondrial localization (Gandhi *et al.* 2006). This protein is involved in cell respiration (Gandhi *et al.* 2009), protein folding and degradation (Moriwaki *et al.* 2008; Dagda *et al.* 2009), and mitochondrial function such as fission/fusion dynamics (Deng *et al.* 2005; Poole *et al.* 2008), trafficking (Weihofen *et al.* 2009), and calcium signalling (Marongiu *et al.* 2009). Further, PINK1 has been shown to protect cells against oxidative stress-induced apoptosis (Deas *et al.* 2009) by phosphorylating the mitochondrial chaperone tumour necrosis factor receptor-associated protein 1 (TRAP1)/heat shock protein 75 (Hsp75) (Pridgeon *et al.* 2007). PINK1 colocalizes and interacts with TRAP1 in the mitochondrial intermembrane space. Upon phosphorylation, TRAP1 prevents cytochrome c release and $H_2O_2$-induced apoptosis by an unknown mechanism, and the ability of PINK1 to phosphorylate TRAP1 is impaired by kinase-inactivating and PD-associated mutations (Pridgeon *et al.* 2007)

PINK1 has been shown to interact with several chaperone proteins, which are known to affect both its stability and its ability to function as a neuroprotective kinase (Deas *et al.* 2009). As a result of PINK1 deficiency in neurons, mitochondria suffer from calcium overload, and this stimulates ROS production in the mitochondria and cytosol (Gandhi *et al.* 2009). PINK1-deficient cells are more susceptible than wild-type cells to apoptosis after exposure to mitochondrial toxins (Deng *et al.* 2005; Haque *et al.* 2008; Wood-Kaczmar *et al.* 2008). In addition, overexpression of the wild-type PINK1 protein protects cells against death mediated by chemical insults such as MPTP, but this effect is abrogated when the protein carries either a PD-associated mutation or a kinase-inactivating mutation (Petit *et al.* 2005; Haque *et al.* 2008).

The majority of mutations in *PINK1* associated with PD are found within the kinase domain. The locations of these mutations, including the one that resides within the ATP binding pocket of PINK1, suggest that loss of PINK1 kinase activity is responsible for disease initiation (Deas *et al.* 2009). Defects in complexes I, II and IV are observed in the striatum of *pink1*-knockout mice (Gautier *et al.* 2008). Inhibition of *PINK1* expression also induces autophagic mitochondrial sequestration, delivery of mitochondria to lysosomes, and a decrease in the cellular levels of mitochondria (Dagda *et al.* 2009). PINK1 inactivation in mice impairs dopamine release but does not alter dopamine levels, the number of dopaminergic neurons, dopamine synthesis, or levels of dopamine receptors (Kitada *et al.* 2007; Gautier *et al.* 2008; Morais *et al.* 2009). The impairment of dopamine release in *pink1*$^{-/-}$mice is sufficient to compromise nigrostriatal circuit function (Kitada *et al.* 2007).

In addition, it was recently demonstrated that PINK1 deficiency causes astrocyte dysfunction including decreased mitochondrial mass and membrane potential, increased intracellular ROS levels, decreased glucose-uptake capacity, and decreased ATP production (Choi *et al.* 2013). Further, organotypic brain slices from *pink1*$^{-/-}$ mice have increased levels of proinflammatory cytokines, IL-1β and IL-6 (Kim *et al.* 2013).

#### *Parkinsonism associated deglycase –DJ-1 or PARK7*

*DJ-1* deletions and point mutations account for ~1–2% of autosomal recessive early onset PD cases (Abou-Sleiman *et al.* 2003; Bonifati *et al.* 2003). Patients with DJ-1-related PD have slow disease progression and respond to treatment with L-Dopa. (Abou-Sleiman *et al.* 2003). Loss of DJ-1 function impairs nigrostriatal dopaminergic function and produces age-dependent and task-dependent motor behavioural deficits (Chen *et al.* 2005; Goldberg *et al.* 2005; Kim *et al.* 2005).

The *DJ-1* gene encodes a small 189-amino-acid protein that belongs to the ThiJ/PfpI family, which includes proteins that function as proteases and chaperones (Lev *et al.* 2006). *DJ-1* is expressed in neurons as well as glial cells in a different brain region (Bandopadhyay *et al.* 2004; Bader *et al.* 2005) and is localized in the cytoplasm, nucleus (Nagakubo *et al.* 1997; Bader *et al.* 2005), and mitochondria (Zhang *et al.* 2005) of the cell. It has been suggested that DJ-1 plays a role in maintenance of mitochondrial

complex I activity (Hayashi *et al.* 2009). The proportion of cells with mitochondrial DJ-1 staining is increased under oxidative conditions, suggesting that oxidation promotes the mitochondrial localization of DJ-1 (Blackinton *et al.* 2005).

The most common mutation in *DJ-1* is L166P, which destroys its protease activity (Olzmann *et al.* 2004) and chaperone activity (Shendelman *et al.* 2004). The L166P mutation impairs the folding of DJ-1 protein, resulting in a spontaneously unfolded structure that is incapable of forming a homodimer with itself or a heterodimer with wild-type protein (Olzmann *et al.* 2004). Further, mutated DJ-1 is not observed uniformly in the cytosol but is mostly localized to the mitochondria (Zhang *et al.* 2005). Additionally, neuronal cells that carry mutant forms of DJ-1 are susceptible to death in parallel with the loss of oxidized forms of DJ-1 (Yokota *et al.* 2003; Taira *et al.* 2004), and neuroblastoma cells with decreased DJ-1 levels are more sensitive to toxins than cells with the wild-type protein (Lev *et al.* 2008).

Cell death induced by hydrogen peroxide treatment is dramatically reduced by overexpression of wild-type DJ-1 but not by expression of L166P mutant DJ-1 (Yokota *et al.* 2003). DJ-1-deficient neurons show a 20% increase in cell death compared with *DJ-1*$^{+/+}$ neurons (Kim *et al.* 2005). Moreover, *DJ-1*$^{-/-}$ mice are more vulnerable to MPTP treatment; a greater loss of dopaminergic neurons is observed in these mice than in wild-type mice upon MPTP treatment (Kim *et al.* 2005).

Overall, the genetic models of PD have been usefull in teasing the mechanisms involving specific gene pathaways that underly the disease pathology. These models (e.g. *parkin*, *pink1* or *DJ-1* knockout mice models) in particular have been useful in understanding the earlier stages of the disease associated with nigrostriatal dysfunction. Barring some exceptions, like the LRRK2 R1441G BAC transgenic mice that recapitulate the progressive age-dependent deficits in motor functions, or TH promotor driven α-Syn (A30P/A53T) transgenic mice that show such defects along with progressive loss of SNpc DA neurons, most genetic models do not produce any overt neurodegeneration or progressive motor dysfunction. This limits their utility in testing neuroprotective therapeutic strategies or in understanding the selective neuronal vulnaribility in PD. Moreover, most of these models do not produce the typical Lewy body pathology, nor do they faithfully represent idiopathic PD. Given these limitations, attempts have been made to produce more robust 'dual hit' models that combine gene mutations and environmental toxins.

## Gene–environment interactions (G × E) in PD, and related models

PD is an age-related neurodegenerative disease, and both genetic defects and exposure to environmental risk factors are linked to the disease. The occurrence of PD-related genetic defects even in healthy carriers, as well as the variable age of onset and variations in disease phenotypes suggest a role for both genetic and environmental factors in its development. The lack of nigral dopaminergic neuronal death in most gene-based PD animal models and the limited amount of α-synuclein-containing Lewy body inclusions in most toxin-based PD models further support the essential role of gene–environment interplay in PD pathogenesis (Gao and Hong 2011).

A few human association studies have examined gene–environment interactions, although many studies have described positive associations between genetic polymorphisms and increased risk for PD (Gao and Hong 2011). Cumulative and interactive effects of cigarette smoking and gene polymorphisms in *iNOS* (Hancock *et al.* 2006) and α-synuclein (McCulloch *et al.* 2008) appear to modulate risk for PD. Moreover, pesticide exposure, including high exposure to paraquat and maneb, in carriers of *dopamine transporter* (*DAT*) genetic variants increased PD risk (Ritz *et al.* 2009). Further, pesticide exposure seems to exacerbate pathogenic effects in α-synuclein-A53T mutant dopaminergic neurons in a human iPSC model of PD (Ryan *et al.* 2013). However, in a study comparing PD patients and controls in five European centres, the majority of gene–environment analyses did not show significant interactions between genes and exposure to solvents, pesticides, or metals (Dick *et al.* 2007).

The dual-hit hypothesis proposes the requirement for at least two independent triggers to happen before the disease manifests (Boger *et al.* 2010). Recently, several animal models to test this dual-hit hypothesis have been developed. An example is administration of MPTP to α-synuclein, Parkin, or DJ-1 transgenic mice; these genetic model mice are more susceptible to toxins, and they recapitulate several pathological processes found in human PD (Manning-Bog and Langston 2007). Similarly, rats that received prenatal exposure to LPS were found to be more susceptible to accelerated dopaminergic neuron loss upon postnatal exposure to rotenone (Ling *et al.* 2004). Moreover, transgenic expression of human α-synuclein increased the vulnerability of dopaminergic neurons to LPS-induced inflammation, which in turn resulted in accumulation of insoluble α-synuclein aggregates in nigral neurons and death of nigral dopaminergic neurons. Interestingly, nitrated or oxidized α-synuclein has been detected in Lewy body-like cytoplasmic inclusions in LPS-injected transgenic mice (Gao *et al.* 2008). Synergistic effects of low-grade neuro-inflammation and α-synuclein dysfunction drive chronic PD neurodegeneration, although neither factor alone was found to be sufficient to cause neuronal death (Gao *et al.* 2011).

Oxidative stress is believed to be a major component of age-related disorders such as cancer, stroke and neurodegenerative diseases. Oxidative stress influences diverse cellular processes and plays a prominent role in the

induction of neuronal death. For instance, excessive free radicals can damage proteins (such as α-synuclein), lipids, DNA, or RNA, leading to cell dysfunction and eventual cell death. The importance of oxidative stress in dopaminergic neurodegeneration elicited by inflammation or various environmental toxins has been demonstrated extensively, and growing experimental findings indicate that all known PD-associated genetic mutation are directly or indirectly related to oxidative stress (Gao and Hong 2011). Damage to multiple genes can have either additive or synergistic effects and mimic the human disease more closely.

## Models testing glia-neuron interactions in PD

Several groups have reported evidence for noncell-autonomous mechanisms in which neurodegeneration is influenced by toxicity or mutant protein expression in both neuronal and nonneuronal cells in the neighbourhood of the vulnerable neurons. These cell types include CNS glial cells: astrocytes (Custer *et al.* 2006; Giorgio *et al.* 2007), oligodendrocytes (Yazawa *et al.* 2005) and microglia (Liberatore *et al.* 1999; Boillee *et al.* 2006), which are in close contact with neurons, although each of them differ in their type of interactions with neurons.

Three different pathways of glial involvement in noncell-autonomous degeneration of the vulnerable neurons have been described. They are: (i) toxicity within neurons stimulates damaging responses from glia that lead to further neurotoxicity not directly attributed to synthesis of the mutant protein in neurons; (ii) mutant protein expression or toxicity in glial cells disturbs a normal glial response, amplifying initial damage to the vulnerable neurons; (iii) mutant protein expression or toxicity within glia disturb normal glial function, thus becoming a primary source of neurotoxicity, potentially independent of mutant (or toxic) effects within the neurons at risk.

Microgliosis in PD, which incorporates an elevation of iNOS, indicates that there may be a microglial role in amplifying the initial damage. This role of microglia is of mechanistic importance in understanding neurodegeneration as the microglial activation precedes the neuronal damage. This is consistent with the observation that *iNOS*-null mice are more resistant to MPTP toxicity than wild-type mice (Liberatore *et al.* 1999; Croisier and Graeber 2006). In further support to the above point, the inflammatory modulator minocycline reduces MPTP neurotoxicity in model systems only in the presence of microglia (Du *et al.* 2001). Thus, in PD, converging damage within astrocytes, microglia, and invading T cells, as well as the target neurons, creates a synergistic and additive effect, which is detrimental to the neurons. This is probably further exacerbated by neuronally restricted oxidative pathways (Ilieva *et al.* 2009; Teismann *et al.* 2003).

## Reprogrammed cell models

### *hiPSC-based PD models*

Even though rodent and nonhuman primate models have been successful in recapitulating many aspects of PD, none, so far can represent all aspects of the disease, especially its progressive nature and the complex aetiology underlying its sporadic form. Moreover, post-mortem brain tissues obtained from PD patients, who are often at a late stage of disease at the time of death, provide only limited information. Therefore, studying PD using model systems that most closely represent the human condition has been challenging until recently.

Recent advances in the stem cell field and the discovery of human induced pluripotent stem cell (hiPSC) technology (Takahashi *et al.* 2007) have provided an unprecedented opportunity to generate patient-specific *in vitro* models to study diseases in a human context. hiPSCs are produced by reprogramming easily accessible human tissues like skin or blood cells to a pluripotent state by the introduction of fate determining 'pluripotency factors'. The original reprogramming method invented by Yamanaka and colleagues (Takahashi *et al.* 2007) was relatively inefficient in producing hiPSCs, and used retroviruses to introduce the master transcription factors OCT4, SOX2, KLF4, c-MYC into human fibroblasts. We and others have subsequently devised protocols that are considerably faster, more efficient and employ integration-free or viral-free strategies to produce hiPSCs (Fusaki *et al.* 2009; Lin *et al.* 2009; Zhou *et al.* 2009; Warren *et al.* 2010; Zhu *et al.* 2010; Ban *et al.* 2011; Okita *et al.* 2011; Hou *et al.* 2013).

A prerequisite for modelling PD using hiPSCs is the availability of methods to generate the relevant cell types, especially the mid-brain (specifically from the A9 region) dopaminergic neurons, that are most affected by the disease. Several protocols have been described to date for the differentiation of hiPSCs to A9 dopaminergic neurons. They take advantage of the instructive capacity of critical transcription factors (e.g., OTX2, LMX1a, FOXa2, LMX1b, MSX1, EN1, NGN2, NURR1 and PITX3) and signalling molecules (e.g., SHH, WNT and FGF 8) that govern mammalian midbrain development (Ono *et al.* 2007). Some of these strategies also include genetic programming of PSCs with DA-inducing transcription factors (Chung *et al.* 2005; Kim *et al.* 2002, 2006) or coculture with stromal feeder cells (Perrier *et al.* 2004; Roy *et al.* 2006). However, most of these methods produced mixed cultures containing TH+ cells whose identity as *bone fide* A9-type DA neurons (defined by coexpression of key transcription factors like LMX1A/FOXA2/NURR1, expression of inwardly rectifying potassium channels (GIRK2), and the capacity to produce pace-maker activity mediated by Cav1.3 calcium channels) was unclear. The current gold standard for producing mid brain DA

**Table 2.** iPSC models for studying PD. The table provides a comprehensive description of the main finding from currently available genetic iPSC models. These hiPSCs were generated either directly from patient samples containg the PD associated gene mutation or by introducing a known PD mutation to hiPSC or ESCs using gene targeting techniques. All these studies focussed on gene mutations that appear in patients with familial forms of PD, and shed light on the mechanisms of disease progression.

| Gene name | hiPSC lines | Main findings | Reference |
|---|---|---|---|
| Autosomal-dominant familial PD | | | |
| *SNCA*/*PARK1* | AST (alpha-synuclein triplication)-unaffected first degree relative control | Higher levels of α-Syn in patient-derived iPSC. | Devine *et al.* (2011) |
| | | Overexpression of markers of oxidative stress, and sensitivity to peroxide induced oxidative stress. | Byers *et al.* (2011); Lin *et al.* (2016) |
| | | α-Syn triplication results in increased ROS production, reduction of endogenous glutathione and subsequent neuronal toxicity. | Deas (2016) |
| | | Impairment in neuronal differentiation and maturation to dopaminergic or GABAergic neurons. | Flierl *et al.* (2014); Hemen-Ackah et al. (2017); Oliveira *et al.* (2015) |
| | WIBR-iPS-SNCAA53T<br>WIBR-iPS-SNCAA53T-Corr | Generate hiPSC and ESC lines that contain α-Syn mutations using ZFN technique. | Soldner *et al.* (2016) |
| | | Differentiate A53T mutant and mutation corrected hiPSC isogenic lines to A9 dopaminergic neurons. Discover a new pathway (MEF2C, PGC1α) and involvement of S-nitrosylation in disease mechanism. | Ryan *et al.* (2013) |
| | | Differentiate A53T mutant and corrected hiPSC to cortical neurons. Demonstrated nitrosative stress, accumulation of ER–associated degradation substrates, and ER stress. | Chung *et al.* (2013) |
| | | Aberrant synaptic connectivity and transcriptional alterations in genes involved in synaptic signalling. | Kouroupi *et al.* (2017) |
| *LRRK2*/*PARK8* | LRRK2-PD-iPSC carrying $LRRK2^{G2019S}$ and $LRRK2^{R1441G}$ mutation-patient derived, non-isogenic controls | Identified *in vitro* phenotypes associated with ID and/or LRRK2 PD, which could be harnessed as readouts for drug screening studies. | Sanchez-Danes *et al.* (2012) |
| | | hiPSCs derived NSCs differentiate less efficiently into dopaminergic neurons and the neurons exhibit defects in branching. | Hammond *et al.* (2007) |
| | | Remarkable increase in COX-2 RNA levels in PD, and a blunted response to a pro-inflammatory stimulus. | Lopez de Maturana *et al.* (2014) |
| | | LRRK2 iPSC derived neurons show abnormal calcium dynamics and neurite aggregations. | Schwab *et al.* (2015) |
| | LRRK2-PD-iPSC carrying $LRRK2^{G2019S}$ with gene corrected isogenic control | Dysregulation of copine VIII, annexin A1, calcium-dependent activator protein for secretion 2, and ubiquitin-like with PHD and ring finger domains 2, Microtubule-associated protein 7, which have not previously been associated with PD and increased activation of ERK. | Reinhardt *et al.* (2013) |
| | LRRK2-PD-iPSC carrying $LRRK2^{I2020T}$ patient derived. | Patient iPSC-derived neurons had a lower phospho-AKT level, activated glycogen synthase kinase-3β (GSK-3β) and high Tau phosphorylation. | Ohta *et al.* (2015) |

**Table 2** (*contd*)

| Gene name | hiPSC lines | Main findings | Reference |
|---|---|---|---|
| Autosomal-recessive, early-onset PD | | | |
| *Parkin*/*PARK2* | Two patients with Parkin mutations (deletions in different regions) | Increased oxidative stress and enhanced activity of the Nrf2 pathway. iPSC-derived neurons, but not fibroblasts or iPSCs, exhibited abnormal mitochondrial morphology and impaired mitochondrial homeostasis. | Imaizumi *et al.* (2012) |
| | T240R mutant Parkin patient derived iPSCs and age matched controls | Loss of Parkin in hiPSC-derived DA neurons increased the transcription of monoamine oxidases and oxidative stress, reduced DA uptake and increased spontaneous DA release. Reduced neuronal process complexity (total neurite length, number of terminals, number of branch points) and decreased microtubule stability in hiPSC-derived neurons from Parkin-mutant PD patient. | Jiang *et al.* (2012) |
| *PINK1*/*PARK6* | Q456X non-sense mutation V170G missense mutation | Cells were differentiated to dopaminergic neurons. Impaired recruitment of lentivirally expressed Parkin to mitochondria, increased mitochondrial copy number, and upregulation of PGC-1α. | Ren *et al.* (2015) |
| | Q456X | Increased production of mitochondrial ROS, and dysfunction in mitochondrial respiration, in hiPSC-derived dopaminergic neurons. | Seibler *et al.* (2011) |
| | V170G | Differences in mitophagy between neuronal and non-neuronal cells. In neurons, no mitophagy is detected. | Cooper *et al.* (2012) |
| | | | Rakovic *et al.* (2013) |

neurons from PSCs is the floor-plate based method, originally described by Lorenz Studer's group (Kriks *et al.* 2011). This robust protocol spanning six weeks generates cultures containing over 80% of TH+ neurons, many of which also exhibit the virtues of A9 phenotype described above (Kriks *et al.* 2011; Kirkeby *et al.* 2012; Ryan *et al.* 2013). Several improvements to this protocol have been recently described, including the use of specific substrates that enhance differentiation and function of the neurons produced (Hyysalo *et al.* 2017).

Another cell type that has a key role in PD pathology is the GABAergic medium-spiny neurons (MSN) of the striatum, which synapse with dopaminergic neurons projecting from the substantia nigra. Protocols have been developed to differentiate these neurons from hiPSCs (El-Akabawy *et al.* 2011), but the neurons so generated have been mainly used so far to study Huntington's disease. More recently, approaches have been developed to make midbrain 3D-organoids (Qian *et al.* 2016), which allows the study of brain development and pathology in a context that resembles more closely their *in vivo* physiology.

In a multifactorial, age-related, and progressive disorder like PD, the genetic background of the individual may play an important role in its pathogenesis. hiPSCs present a major advantage in this context as these cells originate from a patient with the disease. However, this situation also presents a new issue with respect to the type of control that can be used in these studies. Recent advances in gene editing allow for the introduction of gene mutations and deletions, as well as targeted correction of the disease mutations. A variety of techniques are now available to perform such editing, including those utilizing zinc-finger nucleases (ZFN), transcription activator-like effector nuclease (TALEN), and clustered intersperse short palindromic repeats/Cas9 (CRISPR/Cas9) systems (Doudna and Charpentier 2014; Hsu *et al.* 2014). Indeed, the developments in CRISPR/Cas9 based gene editing has dramatically simplified the generation of isogenic hiPSC lines (Cho *et al.* 2013). An isogenic line has an identical genetic background to that of the cognate mutant line, making it an ideal control for the study (Soldner *et al.* 2011; Reinhardt *et al.* 2013; Ryan *et al.* 2013; Shaltouki *et al.* 2015; Arias-Fuenzalida *et al.* 2017; Qing *et al.* 2017).

During the past few years, several groups have utilized hiPSC technology to generate lines from PD patients carrying disease associated gene mutations. DA neurons generated from these mutant hiPSCs show Lewy bodies and Lewy neurites, selective vulnerability to PD associated pesticide exposure, elevated oxidative stress, mitochondrial dysfunction, defective autophagy, and aberrant proteostasis (Cooper *et al.* 2012; Chung *et al.* 2013, 2016; Reinhardt *et al.* 2013; Ryan *et al.* 2013; Soldner *et al.* 2009; Woodard *et al.* 2014; Lin *et al.* 2016). These studies, which are summarized in table 2, have provided critical insights into the disease mechanisms and have enabled novel drug discovery efforts to combat PD (figure 2).

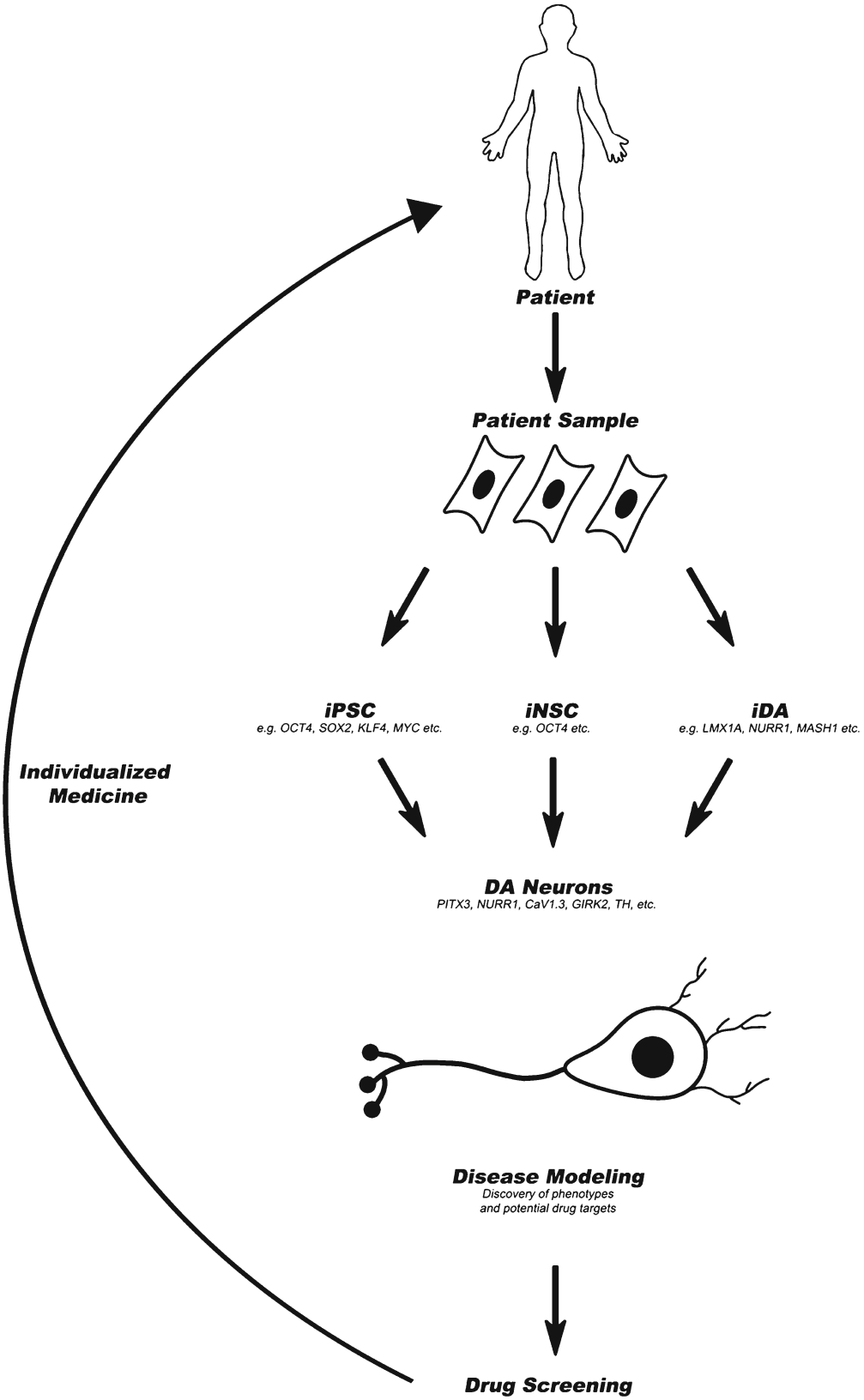


**Figure 2.** Strategies for using patient cells to model PD. Investigators obtain patient and healthy controls samples. The cells are then reprogrammed using various procedures to produce iPSCs or direct reprogrammed neural stem cells (NSCs)/neurons. The DA neurons thus produced can be used to study mechanisms and aetiology of the disease, and for phenotypic characterization and discovery of drug targets. These targets are utilized in drug screening for development of individualized therapies.

Although iPSC technology has revolutionized the way human diseases are modelled, it has several key limitations. For instance, hiPSC lines generated from different individuals, or even between multiple clones from the same individual, can show striking differences in their capacity to differentiate to specific lineages. Moreover, hiPSC work requires sophisticated tissue culture skills, and establishing a line can take up to 3–6 months in a typical stem cell lab. The unavoidable prolonged culturing requirements are also known to introduce unwanted mutations in the resulting lines (Peterson and Loring 2014). More importantly, and relevant to neurodegenerative disorders, the reprogramming process involves epigenetic changes that rejuvenate the cells to an embryonic state, therefore much of the age-associated features critical to disease manifestations in an adult patient may not be preserved in these hiPSC derived neurons.

#### *Direct neural conversion of somatic cells*

The more recent discovery of direct neural conversion (also known as transdifferentiation or direct reprogramming) techniques can potentially resolve most of the drawbacks of hiPSCs discussed above (Kim *et al.* 2012). In the first report of direct neural conversion, Marius Wernig and colleagues used *Brn2, Ascl1* and *Myt1l* to convert mouse embryonic fibroblasts to induced neurons (iNs) (Vierbuchen *et al.* 2010). We have shown that the introduction of a combination of transcription factors and a microRNA (*miR-124, BRN2* and *MYT1L*) into adult human skin fibroblasts can cause their direct neuronal conversion (Ambasudhan *et al.* 2011). Others have also reported similar neuronal conversions from human somatic cells by using various combinations of transcription factors, microRNAs and/or small molecules (Marro *et al.* 2011; Pang *et al.* 2011; Yoo *et al.* 2011; Ladewig *et al.* 2012; Xue *et al.* 2013; Victor *et al.* 2014). Moreover, other groups have produced human induced dopaminergic neurons (hiDAs) by modifying the original reprogramming cocktail with midbrain dopaminergic neuronal lineage specific transcription factors (Addis *et al.* 2011; Caiazzo *et al.* 2011; Kim *et al.* 2011; Pfisterer *et al.* 2011; Liu *et al.* 2012). These hiDAs exhibited characteristic functional attributes of dopaminergic neurons, including electrophysiological properties, gene expression profile and the capacity for dopamine uptake and synthesis with release.

When compared to hiPSC generation, the direct neuronal conversion technique is much faster, usually taking about three weeks to produce iNs from the starting cells. Moreover, there is no pluripotent intermediate stage or cell division involved, mostly preserving in the iNs the age-related features and other cellular attributes of the starting material. Indeed, a recent study reported that the transcriptome signatures and the age-related nucleocytoplasmic defects are more faithfully reproduced in the directly converted neurons when compared to hiPSC derived neurons (Mertens *et al.* 2015).

A major drawback of the direct neuronal conversion is the limited number of neurons generated from the starting population. To circumvent this problem, we and others have reported methods to convert human somatic cells to NSCs, which can then be differentiated to various neuronal cell types (Han *et al.* 2012; Kim *et al.* 2012; Lujan *et al.* 2012; Ring *et al.* 2012; Thier *et al.* 2012; Zhu *et al.* 2014). Despite these remarkable advancements, the direct neural conversion technique has other limitations for disease modelling. These include the low efficiency of conversion, the heterogeneity of the cells produced, the possibility that the converted cells may still retain some of the epigenetic memory of the starting somatic cells complicating the phenotypes produced in the iN cells, and that the observed phenotypes may only represent cell-autonomous effects-unless coculturing of iNs with other glial/neuronal cell types is employed.

## Concluding remarks

In a span of 200 years, since James Parkinson first described PD, we have come a long way in our understanding of the molecular and cellular aspects that underlie this disorder. While we have been successful in modelling some of the critical aspects of the disease in rodents and primates, so far none of these models can recapitulate PD in its totality. This is not so surprising as PD is specific to humans, and the disease per se is very complex and multifactorial. In this context, the reprogrammed cell-based 'human models' are invaluable resources as they provide human context to study PD and present a novel opportunity to approach the disease in a patient-specific manner. However, these hiPSC and hiN models carry all the limitations associated with an *in vitro* system, which can never become a replacement for *in vivo* animal studies. Rather, the different model systems can yield information as various pieces of a puzzle that when put together may provide a more comprehensive view of the disease. PD pathology also seems to follow the recurring theme involving oxidative stress, mitochondrial dysfunction and aberrant proteostasis that defines the cellular pathology of other neurodegenerative disorders like AD or amyotrophic lateral sclerosis (ALS). However, what triggers these cellular events that eventually lead to the systemic defects that manifest in clinical symptoms of PD is still unclear. Moreover, the nonmotor/cognitive manifestations in PD are often overlooked when modelling the disease. In an effort to address such gaps in the field more and more investigators are approaching PD using multiomics and interdisciplinary approaches with a variety of models. These studies, which may yield novel drug targets, along with global cell-therapy initiatives like G-Force PD, offer

considerable promise for discovering a long awaited disease modifying treatment for PD.

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