

Review

Stemming the Hype: What Can We Learn from iPSC Models of Parkinson's Disease and How Can We Learn It?

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Abstract. Parkinson's disease (PD) is a prevalent and debilitating neurodegenerative disorder for which there are no available cures. PD pathogenesis is poorly understood because appropriate animal and *in vitro* models are lacking. The development of induced Pluripotent Stem Cells (iPSCs) has allowed researchers to generate disease-specific dopaminergic neurons *in vitro* by reprogramming skin cells from patients with the disease. It is hoped that this unprecedented access to PD patients' neurons will yield mechanistic insights into PD pathogenesis, a platform for drug screening, and a means of early diagnosis. In this article I critically evaluate the current usage of iPSCs in PD research. I first outline the iPSC paradigm and emphasise the benefits of this approach for modelling PD. I then ask what we can learn from the iPSC-based studies done to date. I argue that these studies have not been particularly informative when considered as an isolated body of evidence. I suggest that the limitations of this technology can be overcome, and I conclude that iPSCs have the potential to be an extremely useful tool in PD research. However, they will never be a panacea and should continue to be used in concert with other *in vitro* and animal models.

Keywords: Disease model, *in vitro*, induced pluripotent stem cells, neurodegeneration, Parkinson's disease, stem cell

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease (AD), affecting approximately 1% of people over 60 [1]. It is a debilitating, progressive condition characterised by motor and non-motor symptoms resulting from the loss of dopaminergic neurons (DAn) from the substantia nigra, and of neurons from other brain regions [2]. As age is the major risk factor for developing PD and the world's population is ageing, this disease is rapidly becoming a public health issue.

And yet there are no cures available for PD, primarily because our understanding of the basic processes that underlie disease progression is inadequate. Progress has been hampered by the paucity of appropri-

ate models. Important insights have come from work on patients' post-mortem samples, pharmacologically-lesioned animals, transgenic animals, and various *in vitro* models, but these systems have not provided us with a clear understanding of PD pathogenesis. Finding models that faithfully recapitulate the early events in human PD is essential for developing effective treatments.

The development of induced Pluripotent Stem Cells (iPSCs) has spawned a new approach to modeling PD. iPSCs can be derived from the somatic cells (e.g., skin cells) of patients and control subjects, differentiated into specific neuronal subtypes, and studied in a dish [3, 4]. This article will ask what iPSC models have taught us about PD and what steps can be taken to get more out of this technology. I argue that iPSC-derived neurons are an exciting prospect for PD research; the initial results are promising, and many of the teething issues with this technology are surmountable. I conclude that, if used cautiously and as a complement to

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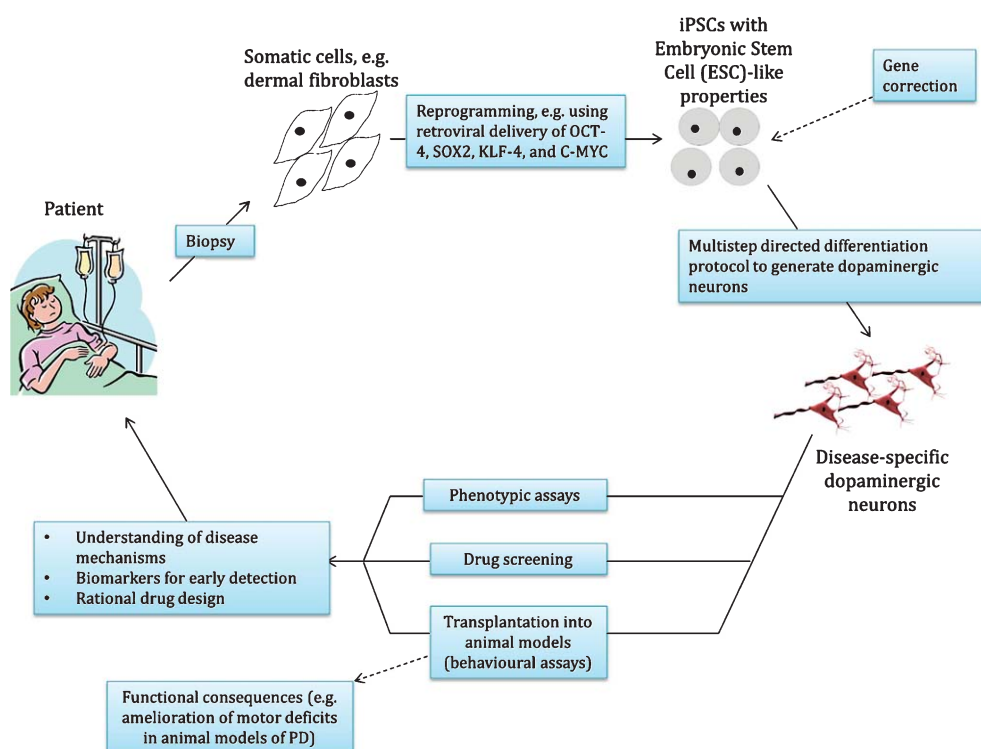


Fig. 1. The iPSC paradigm for modelling PD and other neurodegenerative diseases.

Table 1

Feature of using iPSC-derived neurons	Why is it useful?
Theoretically-unlimited source of patient-specific cells	Cannot extract live neurons from patients. Finite number of neurons obtained from primary culture.
Can generate specific cell types	Can model cell type-specific vulnerability (i.e. of A9 nigral dopaminergic neurons in PD).
Potential for high-throughput assays	Could be used for drug screens (both for general drug discovery and for assessing individual patients' responsiveness to different compounds). Powerful approach for detecting disease phenotypes.
Endogenous levels of gene expression	More physiological relevance than overexpression and transgenic models.
Capture whole disease genome without age- and damage-related epigenetic alterations	Can be used to model earliest stages of disease pathogenesis.
Derived from fibroblasts/somatic cells	Can avoid ethical issues of embryonic stem cell technology.

Table 2

Gene/Protein	Autosomal Dominant/ Autosomal Recessive (AD/AR)
Leucine-Rich Repeat Kinase 2 (LRRK2) [9–13, 36, 38]	AD
α -synuclein (SNCA) [14, 15, 41, 42]	AD
Parkin (PARK2) [16–18]	AR
PTEN-induced putative kinase 1 (PINK1) [11, 19, 20]	AR
Glucocerebrosidase (GBA) [21]	AR

other models, iPSCs will be a useful tool for uncovering the mechanisms of PD pathogenesis, testing therapeutic compounds, and facilitating early diagnosis.

IPSC REVOLUTION

Yamanaka's pioneering experiments demonstrated that somatic cells could be reprogrammed into pluripotency by using retroviral-mediated gene delivery of pluripotent genes OCT4, KLF4, SOX2 and cMYC [3, 4]. iPSCs can theoretically be used to generate unlimited yields of patient-specific neurons or glia *in vitro* for phenotypic assays and/or drug screening (Fig. 1). These cells have several features that make them an attractive system for modeling neurodegenerative diseases like PD (Table 1).

Alternative cellular systems for modelling PD include classical cell lines (such as HEK293 and the neuroblastoma line SH-SY5Y), human Embryonic Stem Cells (hESCs), and primary cultures of neurons from animal models [5]. As iPSCs can be used to generate 'vulnerable' neuronal subtypes (in particular A9 nigral DAN) from patients with the disease, they offer an unprecedented insight into cell-type specific pathology which cell lines and primary cultures cannot match. hESCs can also be differentiated into A9 DAN, however unlike iPSCs they cannot be derived from somatic cells and so are far more limited in their potential applications for research and therapy.

HOPE AND HYPE: THE STORY SO FAR

The first few groups to apply the iPSC technology to neurodegenerative observed no overt disease-related phenotypes in patient-derived neurons [6–8]. More recently, distinct phenotypes have been described in neurons generated from patients with PD, AD, and other conditions [9–36] (Table 3, Supplementary Table 1). Attempts to model PD using iPSCs have focused on familial forms of the disease caused by rare mutations (or multiplications in the case of SNCA) in various genes (Table 2).

What have iPSC-based studies taught us about PD? To answer this question, it is useful to do a thought experiment: if we think of these studies as comprising an isolated body of evidence, what could we learn from it alone? This is inevitably artificial, as progress in PD research relies on a variety of complementary disease models. It is also difficult to synthesise the findings of iPSC-based studies into a coherent whole as individual groups use different differentiation proto-

cols, genotypes, and assays. Nevertheless, asking this question forces us to acknowledge that the results from iPSC-based studies of PD (summarised in Table 3 & Fig. 2) do not yet give us a clear, overarching picture of PD pathogenesis. The interesting and controversial findings of these studies are discussed below.

LRRK2

As LRRK2 mutations are the most common cause of familial PD [37], the function of this protein has been intensely studied in patient-specific iPSC-derived neurons [9–13, 36]. Mutations in LRRK2 have been linked to accumulation of α -synuclein—the main protein component of Lewy bodies [37]—and it is therefore noteworthy that four studies have found increased levels of α -synuclein protein in patient-derived LRRK2-mutant neurons [9, 10, 13, 36]. Accumulation of α -synuclein could be due to increased transcription, increased translation, or decreased degradation. Although Nguyen's group found that SNCA transcription was increased in LRRK2-mutant neurons [9], other groups found no such upregulation [10, 36]. This disparity may be due to a difference in technique (RT-PCR vs. single-cell RT-PCR), neuronal age, genotype, endogenous variability in SNCA expression, or heterogeneity of the neural populations under study. The effect of mutant LRRK2 on SNCA expression is therefore not clear from these studies.

Recent work suggests that mutant LRRK2 could increase α -synuclein levels by disrupting chaperone-mediated autophagy (CMA) – a pathway via which α -synuclein is degraded [13]. This study found that co-localisation of α -synuclein with a lysosomal marker was increased in LRRK2-mutant neurons, suggesting that α -synuclein accumulates at lysosomes when it is not efficiently degraded. However, inhibition of CMA had a more pronounced effect on α -synuclein levels in LRRK2-mutant neurons than controls (in 3 week-old cells), implying that CMA is *more* active in the mutant neurons at this stage [13]. The elevated α -synuclein levels found in the mutant neurons therefore cannot be attributed to defective CMA. Taken together, these studies show that mutant LRRK2 is associated with α -synuclein accumulation in iPSC-derived neurons, but the mechanism for this remains unclear.

Morphological defects have also been observed in LRRK2-mutant patient-specific neurons [10, 13, 36]. In one study, the number and length of neurites was found to be decreased in old LRRK2-mutant DAN—a phenotype that was mimicked by overexpression of

Table 3
Studies reporting disease-relevant phenotypes in patient-derived neurons. NSCs: neural stem cells. NPCs: neural progenitor cells. CNs: cortical neurons

Gene + Ref.	Mutation	Reprogramming strategy	Target cell type	Phenotypes
LRRK2 [10]	Heterozygous G2019S and idiopathic	Retroviral delivery of OCT-4 (O), SOX2 (S), and KLF-4 (K)	MDAn (15–30% FOXA2 ⁺)	1) Increased cytoplasmic α -synuclein protein (LRRK2-mutant only). 2) Morphological alterations in long-term culture 3) Defective autophagic clearance.
LRRK2 [9]	Homozygous G2019S	Retroviral delivery of OSK + C-MYC (M) for control iPSCs	Co-culture with PA6 astrocytes mDAn (<5% yield of FOXA2 ⁺ TH ⁺)	1) Increased total α -synuclein protein. 2) Increased susceptibility to chemical stressors (H ₂ O ₂ , 6-OHDA, and MG-132). 1) Increased susceptibility to various cellular stressors.
LRRK2 LRRK2 PINK1 [11]	Homozygous G2019S (LRRK2), unaffected heterozygous R1441C (LRRK2), and homozygous Q456X (PINK1)	Retroviral delivery of OSKM	>80% CD56 ⁺ neural cells, ~30% neurons, also NSCs and neural crest	2) This phenotype is rescued by a LRRK2 inhibitor and CoQ (an antioxidant), and also by rapamycin in LRRK2 mutant neurons. 3) PINK1 mutants appear more susceptible to oxidative stress than LRRK2 mutants. 4) Abnormal mitochondrial respiration (both LRRK2 and PINK1-mutants).
LRRK2 [12]	Homozygous G2019S	Retroviral delivery of OSK	NSCs	1) Progressive nuclear degeneration. 2) Epigenetic alterations. 3) Defective neurogenesis. 4) Defective differentiation into mature neurons. 5) Increased susceptibility to oxidative stress-induced apoptosis. 6) Targeted correction of the G2019S mutation corrected this phenotype.
LRRK2 [13]	Heterozygous G2019S	Retroviral delivery of OSK	DAn (<i>same culture conditions as [10]</i>)	1) Increased cytoplasmic α -synuclein protein. 2) Overactive CMA in young (3 week) neurons. 3) Underactive CMA in older (75 day) neurons.
LRRK2 [36]	Heterozygous G2019S	Retroviral delivery of OSKM	DAn (~40–50% total TUJ1 ⁺ yield, ~20% TH ⁺ yield)	1) Decreased rate of neurite outgrowth. 2) Decreased basal autophagy. 3) Increased oxidative stress. 4) Increased MAPT expression and tau protein. 5) Increased α -synuclein protein. 6) Increased expression of several novel genes and increased ERK activation. 7) Targeted correction of the G2019S mutation corrected these phenotypes.

Table 3
(Continued)

Gene + Ref.	Mutation	Reprogramming strategy	Target cell type	Phenotypes
LRRK2 [38]	Heterozygous G2019S, unaffected Heterozygous R1441C	Retroviral delivery of OSKM	Neurons (<i>same protocol as [11]</i>) NPCs (<i>yield not quantified</i>)	1) Increased mtDNA damage in both G2019S and R1441C-mutant neurons and NPCs (but not in fibroblasts or iPSCs). 2) Targeted correction of the G2019S mutation corrected this phenotype. 1) Increased α -synuclein mRNA and protein (approximately double). 2) Increased α -synuclein secretion. 1) Increased α -synuclein protein 2) Increased expression of oxidative stress genes 3) Increased susceptibility to peroxide-induced cell death.
SNCA [14]	Heterozygous triplication	Retroviral delivery of OSKM	25–40% of TUJ1 ⁺ neurons are TH ⁺ DAn	1) Increased nitrosative stress (NO levels and protein nitration). 2) Decreased ER-associated degradation of certain substrates (GCase and nitcistrin). 3) NAB2, identified in a yeast screen as a mitigator of α -synuclein toxicity, corrected the nitrosative stress and ER stress phenotypes.
SNCA [15]	Heterozygous triplication	Retroviral delivery of OSKM	6–12% overall yield of TH ⁺ DAn	1) Increased α -synuclein protein and pathogenic oligomerisation. 2) Decreased maximal respiratory rate. 3) Increased susceptibility to toxin-induced cell death.
SNCA [41]	Heterozygous A53T	Lentiviral delivery of OSKM	CNs (<i>yield not quantified</i>)	4) Increased toxin-induced levels of superoxide-MEFC2. 5) Increased basal and toxin-induced NO levels. 6) Decreased transcription of PGC α downstream of MEFC2 – partially rescued by inhibiting NO Synthase.
SNCA [42]	Heterozygous A53T	Lentiviral delivery of OSKM	A9 DAn (>90% total TUJ1 ⁺ yield, of which ~80% are A9 phenotype)	7) MEFC2 activation restored normal PGC α expression and susceptibility to toxins. 1) Synaptic deregulation (increased spontaneous DA release, decreased DA uptake due to decrease in functional DAT levels at cell surface) 2) Increased DA-induced Reactive Oxygen Species (ROS) production 3) Increased MAO expression in.
PARK2 [16]	Heterozygous Exon 3 + 5 deletion, and homozygous Exon 3 deletion	Lentiviral delivery of OSK + NANOG	DAn (<i>yield not quantified</i>)	

Table 3
(Continued)

Gene + Ref.	Mutation	Reprogramming strategy	Target cell type	Phenotypes
PARK2 [17]	Homozygous deletion of exons 2-4, and homozygous deletion of exons 6 & 7	Retroviral delivery of OSKM	Neurons (yield not quantified, some TH ⁺ neurons present)	1) Increased expression of oxidative stress genes. 2) Morphologically-abnormal mitochondria 3) Impaired clearance of damaged mitochondria 4) Alpha-synuclein protein accumulation, correlating with presence/absence of Lewy Body pathology in post-mortem samples.
PARK2 [18]	Compound heterozygous mutant (not affected). Allele 1: partial exon 3 deletion. Allele 2: complete exon 5 & 6 deletion.	Lentiviral delivery of OSK (+C-MYC for controls), or episomal plasmid delivery of OSK+L-MYC+anti-p53 shRNA+LIN28 + NANOG	NPCs (yield not quantified)	1) Increased Manganese-induced ROS production 2) Decreased cytoplasmic Manganese accumulation.
PINK1 [19]	Heterozygous 1366C>T (nonsense), and heterozygous V170G	Retroviral delivery of OSKM	DAn (60% overall TUJ1 ⁺ yield, 11-16% overall TH ⁺ DAn)	1) Decreased recruitment of Parkin to damaged mitochondria. 2) Impaired clearance of damaged mitochondria. 3) Enhanced mitochondrial biogenesis.
PINK1 [20]	Homozygous V170G	Retroviral delivery of OSKM	DAn (~70% overall TUJ1 ⁺ yield, ~10% overall TH ⁺ TUJ1 ⁺ DAn)	1) Loss of mitochondrial depolarisation-induced ubiquitination of mitofusins-2 and parkin. 2) No detectable mitophagy following valinomycin treatment in either control or PINK1 mutant neurons.
GBA [21]	Compound heterozygous mutant. Allele 1: N370S mutation. Allele 2: 84GG insertion.	Retroviral delivery of OSKM	DAn (80% overall TUJ1 ⁺ yield, ~10% overall TH ⁺ DAn)	1) Specific impairment of lysosomal degradation of α -synuclein.

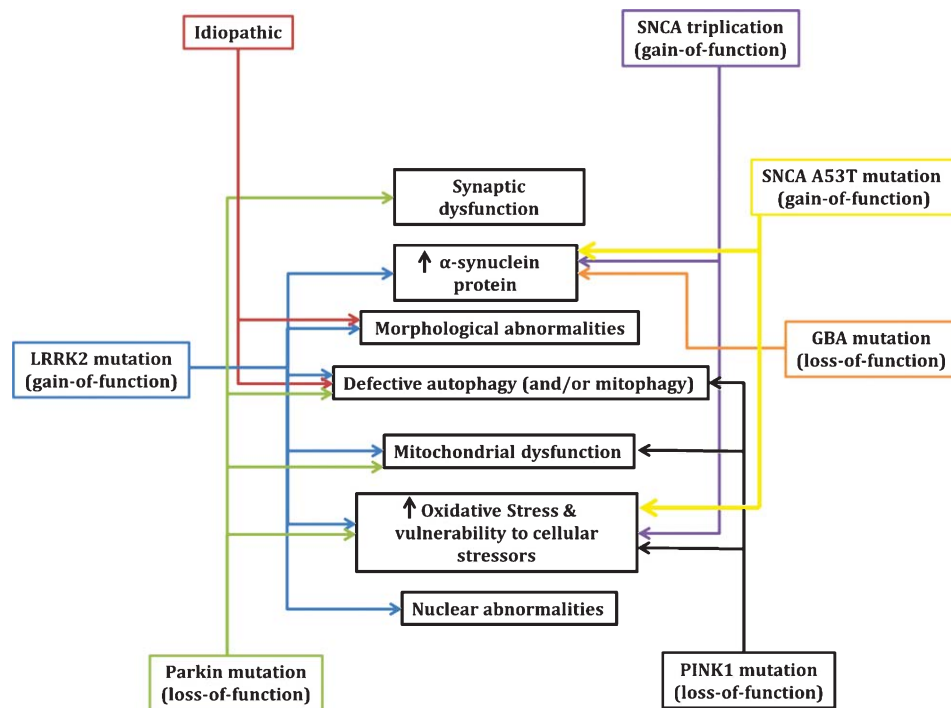


Fig. 2. Phenotypes reported in PD patient-derived neurons.

mutant LRRK2 in control neurons [10]. Another study showed that neurite outgrowth velocity was decreased in LRRK2-mutant DAN, and that this defect could be reversed by targeted correction of the LRRK2 mutation [36]. These results show that mutant LRRK2 disrupts neuronal morphology, although it is unclear how. The mechanism could involve a combination of overactive ERK signaling [36], downstream changes in gene expression [36], and defective autophagy [10].

LRRK2 mutations may exacerbate oxidative stress in neurons. One study found that the expression of oxidative stress-related genes, e.g., Monoamine Oxidase-B (MAO-B) was increased in LRRK2-mutant neurons compared to controls [9]. In addition, older patient-derived neurons show signs of basal stress: the proportion of LRRK2-mutant DAN undergoing apoptosis (indicated by staining for cleaved Caspase 3, CASP3) was found to be higher than for control DAN [10]. These results suggest that LRRK2 mutations increase basal oxidative stress in neurons. Furthermore, LRRK2-mutant DAN show increased susceptibility to cellular stressors [9, 11, 36], although there is some disagreement about the precise susceptibility phenotype. Nguyen's group found that LRRK2-mutant neurons were more susceptible to 6-OHDA, MG-132 (a proteasomal inhibitor), and H₂O₂

than control neurons, whereas Cooper's study found that LRRK2 mutations do not affect susceptibility to these stressors. This discrepancy is probably due to a few factors. Cooper used a shorter drug incubation period (20 hours vs. 48 hours), older cells (56-day vs. 35-day), a different genotype, a smaller maximal dose of H₂O₂, and different readouts for cell stress. Perhaps most importantly, Cooper's study was looking at the whole neuronal culture, whereas Nguyen's study distinguished between TH⁺ and TH⁻ cells.

Oxidative stress and mitochondrial dysfunction are intimately related. Interestingly, Cooper's study found that the basal and maximal mitochondrial respiration rates were lower in LRRK2-mutant neurons compared to controls [11]. On a related note, a recent application of gene-correction technology to iPSC-derived neurons highlighted a causal link between the presence of the G2019S LRRK2 mutation and the accumulation of damage to mitochondrial DNA [38]. By using live-cell imaging and a fluorescent mitochondrial dye, Cooper's study also found that the proportion of mitochondria showing 'random' back-and-forth movement was greater in LRRK2-mutant neurons [11]. Deregulation of mitochondrial mobility might impair neurons' ability to meet local energy requirements and thus may contribute to oxidative stress [39]. An overall picture

is emerging in which the mitochondrion is a central target in LRRK2-mediated pathology.

LRRK2 mutations have recently been shown to impact on nuclear structure and function in iPSC-derived neural stem cells (NSCs) [12]. This study found that expression of mutant LRRK2 was associated with aberrant nuclear morphology and disorganisation of the nuclear envelope in late-passage NSCs. Furthermore, LRRK2-mutant NSCs were more susceptible to MG-132 treatment and differentiated less efficiently into neurons than control NSCs. This study elegantly proved that mutant LRRK2 is responsible for these defects by using an adenovirus-based method to correct the G2019S mutation in the patient-derived iPSCs – this isogenic control line showed none of the mutant phenotypes mentioned. Despite its neatness, this study is not particularly helpful for understanding how mutant LRRK2 causes disease in adult neurons due to the choice of cell-type – the physiological relevance of using NSCs to model DAN for PD is unclear.

SNCA

Mutations and multiplications of the SNCA gene are associated with familial PD [2], and triplications cause a fully-penetrant, particularly aggressive, early-onset form of the disease [40]. Two groups have generated iPSC-derived neurons from patients with the SNCA triplication [14, 15]. Devine's study demonstrated that patient-derived neurons contain at least twice as much α -synuclein mRNA and protein as control neurons [14]. The study by Byers and colleagues also found increased α -synuclein protein in patient-derived neurons, but found no significant difference in SNCA expression between these neurons and the controls [15]. SNCA expression is most probably increased in the SNCA-triplicate neurons – the Devine et al. findings are more convincing as their expression data are normalised to markers of neuronal differentiation to ensure that similar populations of neurons are being compared. A major caveat with this study, however, is that there is no clear demonstration of α -synuclein expression in *dopaminergic* neurons, merely in TH⁺ neurons. It would be helpful to clarify this question in purer cultures of A9 DAN.

What are the functional implications of the SNCA triplication? SNCA-triplicate neurons may be more prone to oxidative stress. Byers and colleagues found that the expression of several oxidative stress-related genes (e.g. MAO-A) was up-regulated in patient-derived neurons, and that neurons derived from one of

the two patients were more susceptible to H₂O₂ treatment than controls. There is therefore some evidence that triplication at the SNCA locus increases oxidative stress in neurons, but because the triplication affects a large region of the genome, we cannot rule out the possibility that this phenotype is caused by genes other than SNCA [14, 15]. Additionally, this phenotype may vary substantially between clones as only two were tested.

Two recent studies have examined the phenotype of A53T-mutant neurons [41, 42]. Using a fluorescent indicator to measure intraneuronal NO and immunostaining to measure levels of protein nitration, Chung et al. found that both of these metrics were increased in A53T-mutant neurons compared to isogenic control lines, suggesting that the SNCA mutation causes a greater degree of nitrosative stress. Higher basal and toxin-induced NO levels were also found in the study by Ryan et al. What is the functional significance of the enhanced nitrosative and oxidative stress phenotype? Ryan's study found that the transcription factor MEFC2 is subject to inactivating modifications by free radicals – a major mechanism appears to be S-nitrosylation of the Cys39 residue in α -synuclein. Additionally, transcription of genes downstream of MEFC2 (including PGC1 α) is decreased in the A53T-mutant neurons – a phenotype which was partially rescued by L-NAME, an inhibitor of Nitric Oxide Synthase (NOS). Incredibly, activating MEFC2 in these neurons using isoxazole almost completely prevented the effects of various mitochondrial toxins such as rotenone. These recent advances are extremely promising examples of how iPSC-derived neurons can be used to pinpoint and manipulate pathological phenotypes in PD.

PARK2 and PINK1

Mutations in PARK2/parkin and PINK1 are associated with early-onset, AR PD [1]. Parkin and PINK1 are thought to form a mitochondrial quality-control system whereby parkin is recruited to damaged mitochondria in a PINK1-dependent manner and subsequently initiates mitophagy [43]. Several studies have used patient-derived neurons to examine how mutations in these genes cause PD.

PARK2 mutations may predispose neurons to oxidative stress [16–18]. One study found that basal levels of reactive oxygen species (ROS), measured with the fluorescent ROS indicator 2',7'-dichlorodihydrofluorescein (DCF), were significantly higher in PARK2-mutant neurons than in controls [17].

In contrast, another group found that PARK2-mutant neurons showed no signs of increased basal oxidative stress, which they measured indirectly by quantifying the protein carbonyl content of the neurons [16].

What seems clear is that these neurons display increased susceptibility to oxidative stress following treatment with dopamine (DA) [16]. This is intriguing as PARK2-mutant neurons show enhanced spontaneous DA release and decreased DA uptake, implying that these neurons experience elevated extracellular DA levels *in vivo*. Another study showed that these neurons produced more ROS than controls following treatment with Manganese – exposure to which is an environmental risk factor for PD [18]. Taken together, these results strongly imply that parkin mutations increase neuronal susceptibility to oxidative stress, but the details of the exact phenotype are still unclear. Similarly, PINK1-mutant neurons are more sensitive than controls to a variety of stressors (Table 3) [11]. Intriguingly, this enhanced sensitivity is abolished by treatment with a LRRK2 inhibitor, suggesting that LRRK2 may be involved in neuronal death resulting from mutations in PINK1.

The phenotypes of PARK2 and PINK1-mutant neurons lend support to the idea that these proteins function together to mediate effective clearance of damaged mitochondria. Impaired mitophagy has been observed in PARK2-mutant neurons, albeit indirectly [17]. Imaizumi et al. found that, following mitochondrial depolarisation, the total inner mitochondrial membrane area was decreased in control, but not PARK2-mutant neurons. Seibler et al. demonstrated that mitochondrial depolarisation triggered recruitment of parkin to mitochondria in control but not PINK1-mutant DAN – a defect that was abolished by overexpressing wild-type (WT) PINK1 [19]. Furthermore, mitochondrial depolarisation led to a decrease in the mitochondrial DNA copy number (a measure of the number of mitochondria) in control but not PINK1-mutant DAN. These results suggest that PINK1-dependent recruitment of parkin to mitochondria is essential for effective mitophagy, and that this system is disrupted in neurons by mutation of either of these genes. However, parkin was overexpressed in Seibler's study and it is possible that the observed differences in mitophagy between mutant and control neurons might be an artefact reflecting variable transfection efficiency. In this vein, a recent study [20] found that endogenous parkin is insufficient for triggering mitophagy in iPSC-derived neurons, calling into question the validity of Seibler's and Imaizumi's methods for assessing mitophagy.

CHALLENGES AND OPPORTUNITIES

iPSC-based models of PD have not lived up to their full potential. Patient-derived neurons display some interesting and relevant phenotypes, but they have not yet yielded any truly original insights into the mechanisms of PD, nor do they seem like a reliable platform for drug screening or diagnosis in their current form. What is holding this work back, and how can these limitations be overcome?

First, iPSC lines display considerable phenotypic variability that is unrelated to their genotype. This 'inter-clonal variability' makes iPSCs an inherently noisy system to work with. Detecting subtle disease-relevant phenotypes with iPSC-derived neurons is therefore a major challenge – the 'signal-to-noise' ratio in the system is very low. By generating multiple lines from each patient and only comparing lines that differentiate with similar efficiency, this variability can be controlled [14]. Developing more efficient differentiation and reprogramming strategies will also help to remedy this problem.

Second, the genetic and epigenetic profile of iPSC-derived neurons may not accurately resemble that of mature neurons. iPSCs commonly display genetic aberrations (e.g. copy number variations [43, 44], somatic coding mutations [45], and chromosomal defects [46]), and epigenetic abnormalities (e.g., X chromosome-reactivation [47] in female cells and transcriptional 'memory' of the cell type from which they are derived [48–51]). The functional impact of these abnormalities on the physiology of iPSC-derived neurons is unclear, although it seems reasonable to assume that, given their frequency, they will have some impact on cell function. A related concern is that *in vitro* differentiation protocols may not produce neurons with a mature phenotype. The most practical solution to these problems is to implement rigorous criteria for selecting which iPSC and iPSC-derived neuronal lines to experiment on, e.g. those detailed in Fig. 3.

Third, in the majority (~97%) of cases PD is not monogenic, but instead results from the interplay of environmental factors and multiple genetic susceptibility loci [2]. Understanding disease pathogenesis in these so-called *sporadic* or *idiopathic* cases is therefore essential. But almost all iPSC-based models of PD have focused on familial forms of the disease [9, 11–21, 36]. Study of these rare forms may shed some light on the pathogenesis of sporadic PD, e.g. by highlighting common phenotypes that result from different genetic defects (see Fig. 2). However, a far more direct approach is required to fully understand how gene-

environment interactions cause PD in sporadic cases. More work should be done using iPSC-derived neurons from sporadic PD patients and from unaffected subjects carrying PD-associated mutations. This avenue of inquiry has already proven fruitful for sporadic PD [10] and sporadic AD [23].

Fourth, given that variations at multiple genetic loci influence the risk of developing PD, great care must be taken when selecting control subjects for iPSC-based studies [2]. To isolate the effect of a rare mutation, e.g., the G2019S substitution in LRRK2, the most adequate control would be an isogenic line with the mutation repaired but exactly the same genetic background. Proof-of-concept studies have shown that Zinc Finger Nucleases (ZFNs) [52] and Transcription Activation-Like Effector Nucleases (TALENs) [53] can be used to correct disease-causing mutations in iPSCs, and to introduce these mutations into control lines. Genome-editing has already been elegantly used to establish a causal relationship between genotype and phenotype in iPSC-derived neurons from PD patients [12, 36, 38, 41, 42] and Huntington's disease patients [25]. Reinhardt et al. demonstrated the power of this approach by discovering a variety of new phenotypes caused by the mutant LRRK2 protein that could not be detected by comparing neurons derived from patients and control subjects [36].

A further issue is that current differentiation protocols generate heterogeneous neural populations, often with low (<10%) yields of DAn. To fully understand why PD pathology has such a predilection for A9 nigral DAn, we need better methods of isolating this neural subtype. One solution is to tinker with the patterning factors used during differentiation, e.g. by overexpressing midbrain transcription factors such as LMX1A [54]. New differentiation protocols can achieve an impressive yield of A9 DAn [42]. Alternatively, Fluorescence Activated Cell Sorting (FACS) can be used to purify neurons, as has been done for iPSC-derived neurons from patients with AD [23] and Amyotrophic Lateral Sclerosis [33]. Another strategy is to bypass the iPSC stage altogether and directly convert skin fibroblasts into DAn [55, 56]. At present, the efficiency of direct conversion is too low to justify its use over iPSC-derived neurons, as iPSCs have the advantage of being self-renewing and thus an unlimited source of cells. Additionally, both the iPSC and direct conversion approaches are based on bombarding cells viral vectors carrying transcription factors. It is possible that viral reprogramming impacts on the genome in an as yet unspecified way, and thereby might distort the biology of cells derived in this way. Non-integrative technolo-

gies (such as episomal vectors, Sendai virus, mirRNA, and mRNA) can offer a complementary, alternative system to bypass this issue.

In a sense an opposite concern is that PD pathogenesis may involve interactions between neurons and glia [57] which cannot be modelled by generating neurons alone. The simplest solution to this problem is to generate patient-specific glial cells in a similar manner, e.g. as was done with astrocytes to study HD [30] and microglia to study Frontotemporal Dementia [35]. Careful co-culture of patient-derived neurons with patient-derived glia will no doubt prove extremely useful as a system for modelling neuron-glia interactions. Steps have already been taken in this direction by Henderson and colleagues who showed that iPSC-derived astrocytes promoted hESC-derived motorneurons *in vitro*, providing a fascinating platform for ALS research [58].

There is also skepticism about whether PD, which usually takes >60 years to develop *in vivo*, can be modelled *in vitro* over a timescale of less than a year. Maintaining neurons for longer *in vitro*, e.g. by coculturing with mouse astrocytes [10], sidesteps this problem to an extent. Another solution might be to combine iPSC-derived neurons with animal models; grafting these cells into rats or mice would allow for long-term analysis of their physiology *in vivo*, as has already been done for PD [59] and HD [28].

An interesting question is whether iPSC-derived neurons could ever be a feasible diagnostic and clinical tool. Could somatic cells be routinely harvested from patients with neurodegenerative disease, reprogrammed into the cell type of choice, and used to inform treatment? This 'personalised iPSC paradigm' could work either by facilitating an accurate pathological diagnosis of different disease subtypes (which may be associated with different responses to certain treatments) or by acting as a platform for drug screens. The equipment and labour costs of the iPSC approach mean that direct clinical applications of this technology on a grand scale are still some way off. However, if costs can be driven down (e.g., by optimizing differentiation protocols) and if more work is done on therapeutic applications of iPSCs (e.g., adapting iPSC-derived neurons for rapid compound screens), these cells may well find their way into clinical practice in the future.

OUTLOOK

The use of iPSC-derived neurons to model PD is still in its infancy. Critics of this technology rightly

point out that it is a highly ambitious project to attempt to model *in vitro* any disorder with complex aetiology. Nonetheless, iPSCs hold great promise for PD research. Armed with a combination of non-integrating reprogramming strategies, stringent cell-selection criteria, better differentiation protocols, neuronal purification techniques, genome-editing strategies, *in vivo* transplantation, international iPSC banks [60], and high-throughput screening of compounds, researchers will be able to harness the full potential of iPSC-derived neurons. Despite its shortcomings, the iPSC technology is an invaluable weapon in the arsenal of those trying to understand the molecular events at the heart of PD pathogenesis.

CONFLICT OF INTEREST

The authors have no conflict of interest to report.

SUPPLEMENTARY MATERIAL

Supplementary material is available in the electronic version of this article: <http://dx.doi.org/10.3233/JPD-130268>.

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