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# Gene-based Therapies in Parkinson's Disease

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**Abstract** Parkinson's disease (PD) is a progressive neurological disorder characterized primarily by the degeneration of nigrostriatal dopaminergic neurons and diminution of the neurotransmitter dopamine. Though dopamine replacement therapies such as levodopa can improve the symptoms of PD, the benefits may be overshadowed by side effects and the onset of symptoms not responsive to dopaminergic treatments (e.g., autonomic symptoms, gait and balance problems, and cognitive impairment). Furthermore, no therapies have proven to slow the neurodegenerative process. Novel approaches to address these difficult problems, and others, are being sought. Over the last decade, several innovative gene therapies for PD have entered human clinical trials in an effort to address both symptomatic and potential disease-modifying effects. Though the results of these trials have been mixed, the therapies have generally been safe and well-tolerated, suggesting gene therapy may be a viable treatment for PD in the future. This article will review past and current clinical trials of gene therapies for PD. In addition, novel preclinical approaches to gene therapy for PD will be described.

**Keywords** Parkinson's disease · Gene therapy · Clinical trials · Neurodegeneration · Dopamine

## Introduction

Gene therapy involves the use of a gene (or, more broadly, genetic material, including DNA and RNA) as an agent to alter cellular/biological function and treat disease. Though

traditionally gene therapy has been primarily thought of as a means for correcting a genetic defect, many other gene-based therapeutic strategies for disorders that are not primarily genetic in origin [such as Parkinson's disease (PD)] have been conceived and tested. These approaches may be particularly advantageous in neurological diseases, as they provide a means for targeting specific molecular pathways and brain regions [1].

Clinical gene therapy can be classified into 1 of 2 categories: 1) *ex vivo* gene therapy, in which cells are genetically modified in culture to express a desired protein or proteins and then transplanted into the patient; and 2) *in vivo* gene therapy, in which genetic information is directly inserted into the patient's own cells. Though *ex vivo* gene therapy strategies may have a role in the treatment of PD [2–4], to date, all human clinical trials of gene therapies for PD have been conducted with the *in vivo* method, utilizing viral vectors to introduce specific genes into the patient's own neurons.

Gene therapy, however, carries specific risks that need to be considered before conducting human clinical trials. One risk is the uncontrolled overproduction of the targeted protein resulting in adverse effects. Gene promoter regions could, theoretically, be used to control gene expression [5], but these approaches have not been tested in human clinical trials. Another potential risk of *in vivo* gene therapy is insertional mutagenesis, in which the introduced gene inserts into the host genome at a site promoting oncogenesis causing neoplastic transformation of the host cell [6]. This risk can be mitigated by the use of viral vectors known to have a relatively low risk of causing this phenomenon [7], but, nonetheless, it remains a concern. Finally, gene therapy may induce an autoimmune and inflammatory response. For example, a gene therapy trial utilizing an adenoviral vector for the treatment of ornithine transcarbamylase deficiency resulted in a fatal inflammatory response in one patient [8]. Though this risk can also be mitigated by the use of certain viral vectors, monitoring for

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immune and inflammatory responses during gene therapy clinical trials is commonly undertaken.

### Gene Delivery Vectors

Advances in the understanding and construction of viral and nonviral vectors for gene transfer have enabled gene therapy to become a realistic form of treatment for many neurological diseases, including PD. The 2 most commonly used viral vectors are lentivirus and adeno-associated virus (AAV). Historically, herpes simplex virus and adenovirus have also proven to be useful, but concerns of resulting inflammatory responses and toxicity have since led to their abandonment [1]. Removal of the genes responsible for virus replication has lowered the risk of secondary immune reactions with the AAV vector, particularly when used in the brain [9–11]. Because the vector is essentially devoid of the viral genome, the large majority of viral DNA remains episomic and is not incorporated into the host genome, thereby additionally reducing the risk of insertional mutagenesis. Though the size of the AAV vector restricts the gene constructs that it can deliver, the aforementioned advantages have led to its near-dominance in human clinical gene therapy trials, particularly in PD. Larger gene constructs can be accommodated by a lentivirus for transduction in dividing and nondividing cells. Generated from a pathogenic retrovirus, the lentiviral vector integrates into the host genome and may produce longer-term transgene expression. Importantly, targeted neurons of the lentiviral vector are typically postmitotic, which may limit the risk of insertional mutagenesis. Regardless of the vector used, however, a major problem in gene therapy for neurological disorders is delivering the gene across the blood–brain barrier (BBB); neither lentivirus nor AAV vectors can cross the BBB, so all human gene therapy clinical trials in PD to date have involved the direct infusion of the viral vector into specific targets in the brain via a craniotomy.

Several nonviral methods of gene delivery are being investigated in preclinical models. For example, it may be possible to transfer genetic material efficiently utilizing a gene gun or electroporation [12]. The former achieves direct gene delivery into tissues or cells by injecting gold particles coated with DNA, which can permeate into the nucleus of the cell [13]. Similarly, electroporation utilizes a method for enhancing cell permeability to improve gene delivery by applying controlled electric fields after the genetic material has been injected [13]. An alternative to direct injection is administering genetic material intranasally, which may allow access to the central nervous system (CNS) and bypass the technical problem of getting gene therapy across the BBB [14, 15]. Genetic information can also be transferred via liposomes which, once coated with polyethyleneglycol (PEG), are stable in blood and can be further modified for active transport into the CNS [16, 17].

These PEGylated immunoliposomes may provide additional utility in that they can remain episomal and thus reduce the risk of insertional mutagenesis, though the degradation of exogenous genetic material may require repeat treatments [18].

### PD Gene Therapy Clinical Trials

Five PD gene therapies have been tested in early phase human clinical trials (see Table 1), and all of them have utilized the AAV or lentivirus vectors for gene transfer; these trials have included therapies aimed at both symptomatic and disease-modifying effects. The symptomatic approaches have focused on either increasing dopamine production by transducing genes involved in neurotransmitter synthesis or normalizing basal ganglia circuitry by altering the neuronal phenotype [19]. With regard to disease modification, several trials have been conducted utilizing a gene for a neurotrophic factor (Neurturin) in an attempt to increase dopaminergic nerve terminals.

#### AAV-Glutamic Acid Decarboxylase

In PD, the loss of dopaminergic nigrostriatal neurons results in downstream changes in basal ganglia circuitry, including decreased gamma-aminobutyric acid (GABA)ergic input into the subthalamic nucleus (STN). GABAergic drugs infused into the STN can improve symptoms in PD patients [20]. Over the last 10 years, 2 early phase clinical trials have investigated a gene therapy approach for increasing GABAergic tone in the STN. This treatment utilized an AAV vector for delivery of the gene for glutamic acid decarboxylase (GAD), the rate-limiting enzyme for the synthesis of GABA, to the STN. The goal of this therapy is to regulate STN firing rates thereby improving the motor features of PD [12].

The first human trial of *in vivo* gene therapy for a neurodegenerative disorder evaluated the safety and tolerability of AAV2–GAD gene therapy in a phase 1, open-label, dose-escalation study in 12 moderately advanced PD patients [21]. All patients had Hoehn and Yahr stage 3 or greater PD, and were considered candidates for deep brain stimulation surgery on the basis of having intolerable motor complications of levodopa. Each patient received 1 of 3 AAV2–GAD doses, which were injected unilaterally into the STN, contralateral to the more clinically impaired side. During 12 months of follow-up, no treatment-related adverse events and no immune responses were observed. Improvements in total motor Unified Parkinson's Disease Rating Scale (UPDRS) scores were seen in both the on and off states after 3 months and persisted for the duration of the study. In addition, metabolic imaging data acquired using [<sup>18</sup>F]fluoro-deoxyglucose positron emission tomography (PET) revealed a treatment-mediated reduction in brain metabolism in the pallidum and

**Table 1** Gene therapy clinical trials in Parkinson's disease

Gene	Viral vector	Target	Study phase	n	Primary outcome measure	Study duration	Baseline H&Y (UPDRS)	Baseline LD therapy <sup>a</sup> (LD eq.)	Ref.
<i>GAD</i>	AAV2	STN	1	12	Safety	12 months	≤ 3 (39.2)	1083 (1402)	[21, 22]
			2	44	UPDRS	6 months	2.8 (37.2)	891.8	[24]
<i>AADC</i>	AAV2	Putamen	1	10	Safety	6 months	3–4 (38.6)	771 (1050)	[26, 27]
<i>TH + GCHI + AADC</i> (Prosavin)	Lenti-virus	Striatum	1	15	Safety	12 months	3–4 (20–60)	U/A	[31]
			2	15	UPDRS	6 months	U/A	U/A	[32]
<i>Neurturin</i> (CERE-120)	AAV2	Putamen	1	12	Safety	12 months	3.1 (42)	(1783)	[45]
			2a	58	UPDRS	12 months	3.0 (38.8)	715.1 (1067.6)	[46]
<i>Neurturin</i> (CERE-120)	AAV2	SNc and putamen	1	6	Safety	15–24 months	2.8 (38.2)	U/A	[47]
			2b	51	UPDRS	15–24 months	≤ 3	U/A	[48]
<i>GDNF</i>	AAV2	Putamen	1	24	Safety	5 years	3–4 (≥ 30)	U/A	[49]

*H&Y* Hoehn and Yahr; *UPDRS* Unified Parkinson's Disease Rating Scale (off-state motor score); *LD* levodopa; *LD eq.* levodopa equivalent dose; *GAD* glutamic acid decarboxylase; *AADC* aromatic L- amino acid decarboxylase; *TH* tyrosine hydroxylase; *GCHI* guanosine triphosphate cyclohydrolase 1; *GDNF* glial cell-derived neurotrophic factor; *AAV* adeno-associated virus; *STN* subthalamic nucleus; *SNc* substantia nigra pars compacta; *U/A* unavailable

<sup>a</sup> mg daily

the thalamus on the operated side [22]. Improvements in motor UPDRS ratings were associated with increases in metabolism in the premotor cortex of the operated hemispheres, suggesting that STN gene therapy altered the activity of the motor cortico-striato-pallido-thalamo-cortical circuit in a manner that would be expected to improve motor function [23].

The highest AAV2–GAD dose ( $1 \times 10^{12}$  vg/ml) was subsequently tested in a phase 2 randomized, double-blind, sham surgery-controlled trial [24]. Forty-four patients with progressive, levodopa-responsive PD were studied; 21 patients were randomized into the AAV2–GAD group and 23 patients into the sham group. Study treatment was infused bilaterally into the STN for both groups; the sham group received infusion of intradural sterile saline (no brain penetration) in place of AAV2–GAD. No major adverse effects related to the operation or gene therapy treatment were reported by any patients during the study. Over the course of the 6-month study, the AAV2–GAD group demonstrated improvement in UPDRS motor scores compared with the sham group (AAV2–GAD 23 % vs sham group 13 %). This treatment effect remained significant at 12 months (unpublished data). Other clinical measures also suggested improvement in the AAV2–GAD group, including measures of consistency of medication effect and freezing of gait. Importantly, the absence of significant adverse events related to AAV2–GAD was also sustained through 12 months of follow-up.

#### AAV–Aromatic L-Amino Acid Decarboxylase

Pharmacological treatment of PD with oral administration of levodopa remains the gold standard symptomatic therapy for PD. However, over time, the beneficial effects of levodopa can be complicated by motor fluctuations and dyskinesias.

Continuous dopamine stimulation may counterbalance these negative long-term effects of drug use [25], prompting the investigation of gene therapy approaches to improve levodopa metabolism.

One such symptomatic gene therapy approach for PD focuses on improving dopamine replacement therapies by enhancing the efficiency of levodopa conversion to dopamine. Aromatic L-amino acid decarboxylase (AADC) is the enzyme that converts endogenous or pharmacologically administered levodopa to dopamine. In PD, however, AADC activity diminishes with the progressive loss of nigrostriatal neurons, further limiting endogenous dopamine levels and leading to the need for larger dosages of levodopa in advancing disease [1]. Transduction of intrinsic striatal neurons with the *AADC* gene can enhance dopamine synthesis and may improve the efficacy of levodopa treatment. Moreover, if the dose of levodopa needed to achieve therapeutic benefit can be decreased, this could lead to a reduction in associated side effects.

An early phase human dose-ranging clinical trial assessing the safety and efficacy of utilizing an AAV2 vector to transduce striatal neurons with the *AADC* gene has been completed [26, 27]. Ten patients received bilateral posterior putaminal infusion of either low- or high-dose AAV2–AADC, in conjunction with orally administered levodopa. No significant adverse effects related to the viral vector were reported; however, 3 patients experienced intracranial hemorrhage from surgery. Only 1 of these patients was symptomatic, but he made an almost complete recovery. Improvements in total and motor UPDRS scores were observed in both the on and off states during 6 months of follow-up. PET imaging using the AADC tracer [<sup>18</sup>F]fluoro-*L*-m-tyrosine revealed an increase in striatal AADC activity at 6 months postinfusion, and these

changes correlated with clinical improvements. The improvement in both off and on state UPDRS scores suggests that AADC gene therapy may enhance the conversion efficiency for both endogenous and orally administered levodopa. The effective dose of levodopa decreased for all patients in the high-dose group and 3 patients in the low-dose group [27]. These promising results will need to be confirmed in a randomized, double-blind clinical trial.

#### Tyrosine Hydroxylase/AADC/Guanosine Triphosphate Cyclohydroxylase

A similar symptomatic therapy has been developed that aims to increase striatal dopamine not only by restoring AADC activity, but also by further increasing endogenous levodopa in dopaminergic and nondopaminergic neurons [28–30]. This approach utilizes 3 genes involved in the production of dopamine: *AADC*, *tyrosine hydroxylase (TH)*, and *guanosine triphosphate cyclohydroxylase (GCH)*. TH and GCH catalyze the synthesis of levodopa from dietary tyrosine, which can then be converted to dopamine via AADC. The potential value of this treatment has been demonstrated in 6-hydroxydopamine (6-OHDA) rodent and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) primate PD models. Utilizing triple intra-striatal transduction with 3 AAV vectors separately encoding AADC, TH, and GCH, improvements in dopamine concentrations were observed leading to the development of a single lentivirus vector to transduce genes for all 3 enzymes. This triple gene approach [Lenti-TH-AADC-GCH (ProSavin)] has been demonstrated to increase extracellular striatal dopamine concentrations in animal models of PD [24]. In addition, long-term expression of the dopamine-modifying genes in the striatal neurons may be achievable with this method as lentivirus-delivered genes integrate into the host genome. The goal of this therapy is to provide more continuous (less pulsatile) dopamine delivery targeted to the motor portion of the striatum, perhaps reducing the risk of levodopa-associated complications such as dyskinesias and hallucinations.

After successful demonstration in animal models [30], ProSavin has advanced into early phase human clinical trials. The initial open-label, dose-escalation study tested 3 doses in 15 moderate-to-advanced PD patients [31]. All patients had Hoehn and Yahr stage 3 or greater PD, and were considered candidates for deep brain stimulation surgery on the basis of having intolerable motor complications of levodopa. No serious adverse events attributable to the study treatment or evidence of immunotoxicity were reported in any of the patients at the 6-month primary efficacy endpoint or in long-term, 36-month follow-up. In addition, significant improvement in motor function relative to baseline was reported in all cohorts at 6 months, with corresponding reduction or stabilization of effective levodopa dose, though these findings have not yet

appeared in a peer-reviewed journal. This early phase study has been extended for long-term follow-up to further assess the safety and tolerability of ProSavin treatment. In addition, a preclinical study is reportedly underway to optimize the effective drug dose prior to testing in a randomized, placebo-controlled human clinical trial [32].

#### AAV-Glial-derived Neurotrophic Factor

Gene therapy has also been utilized to deliver therapies aimed at slowing disease progression and restoring neuronal function (i.e., disease-modifying therapies). To date, these approaches have utilized genes for neurotrophic factors to support the function and survival of nigral dopaminergic neurons. In particular, glial cell line-derived neurotrophic factor (GDNF) is a neuron-type specific growth factor that has been studied extensively for its potential application to PD [33]. Preclinical studies in PD animal models have demonstrated that the direct injection of GDNF into the striatum may be a safe and effective treatment aimed at reducing nigrostriatal dopaminergic cell death, in addition to promoting dopamine axon sprouting near the site of delivery [34–38]. These promising preclinical observations, however, have not been replicated in human clinical trials of direct GDNF injection [39–41], raising the concern that delivery of GDNF may need to be more continuous and better targeted in PD patients. This has led to the investigation of viral vector-mediated delivery of GDNF genes as an alternative to GDNF infusion. Several *in vivo* preclinical studies have successfully evaluated GDNF gene delivery in primate PD models using adenovirus-, lentivirus-, and AAV-based vectors to transduce striatal cells [42–44]. Results from these studies support a beneficial effect of GDNF expression on nigrostriatal degeneration and related motor deficits.

The most extensively studied gene therapy of this type in PD has been the gene for the GDNF family member, *Neurturin (NTN)*. Intra-striatal injections of an AAV2 vector encoding NTN in both MPTP primates and 6-OHDA rodent models of PD have demonstrated the treatment to be safe and effective [1]. These preclinical results led to a phase I, open-label, clinical trial to test the safety and efficacy of bilateral intraputaminatal injection of NTN at 2 dose levels [45]. Twelve PD patients were treated and showed a significant clinical improvement (36 %) in off-medication motor UPDRS scores at 1 year, though [<sup>18</sup>F]Fluorodopa PET imaging did not reveal significant increases in dopaminergic nerve terminals after 12 months. Importantly, the treatment was safe and well-tolerated, without serious adverse events. A subsequent randomized, double-blind, sham surgery-controlled clinical trial was conducted with 58 PD patients [46] in an effort to validate the observed efficacy in the open-label trial. Unfortunately, patients who received NTN did not show significant improvement in the primary outcome measure of off-state motor UPDRS scores at 12 months. However, in a *post hoc* analysis,

a modest, but significant, improvement was observed in the 19 NTN-treated patients who continued blind to an 18-month evaluation, suggesting that perhaps a longer observation period was needed to achieve a benefit. Postmortem analysis of 2 deceased patients demonstrated NTN expression in the putamen, but not in the substantia nigra, indicative of restricted retrograde transport of the AAV vector.

Because of these factors, a second clinical trial was undertaken to directly target the substantia nigra, inject a higher dose to the putamen, and provide clinical follow-up for 3 years. A phase 1, open-label study in 6 PD patients demonstrated the tolerability of treatment with CERE-120 delivered to both the substantia nigra pars compacta and putamen, and, in addition, there were no safety complications in 2 years of follow-up [47]. Based on these data, a phase 2b, double-blind, sham surgery-controlled trial was undertaken to assess the efficacy of combined intraputaminal and intranigral gene delivery of CERE-120 in PD patients. Pre-publication press reports, however, indicate that this trial has failed to demonstrate efficacy [48]. At 15 months, the primary endpoint (off-state motor UPDRS scores) was not improved, though there were significant improvements in some secondary endpoints, including off time measured with diaries. Importantly, the therapy was found to be safe and well tolerated.

Other AAV2–GDNF gene therapies are also being studied for PD, though enthusiasm for this approach may be diminished after the disappointing results for NTN. Currently, an open-label dose-escalation study of AAV2–GDNF delivery in advanced PD patients is enrolling patients to test the safety, tolerability, and efficacy of 4 doses delivered bilaterally to the putamen [49]. Patients will undergo clinical evaluations regularly for 5 years post infusion, in addition to laboratory studies, neuropsychological testing, and neuroimaging. Recent studies have raised the possibility that GDNF may not be effective on dopamine neurons with increased levels of alpha-synuclein (SNCA) [50], suggesting that this therapeutic approach may be only effective for patients with relatively mild PD, and perhaps providing an explanation for the negative clinical trials to date.

## Preclinical Gene Therapy Approaches to PD

### AAV–TH–GCH

Analogous to the triple therapy approach currently used in clinical trials utilizing a lentivirus vector, the development of an AAV vector capable of delivering TH and GCH is also being pursued. Recently, co-expression of *TH* and *GCH* genes on a single AAV vector has been achieved and has been tested in hemiparkinsonian rats and nonhuman primates [51]. A dose-dependent functional recovery associated with

enhanced levodopa production was observed following treatment in unilateral 6-OHDA-lesioned rats. However, in a small cohort of animals, loss of neurons in the globus pallidus resulted from treatment. Further investigation revealed that this cell loss was most likely associated with levodopa production and its downstream effects on pallidal cells. Moreover, it was determined that the dose of the TH–GCH1 vector can be adjusted to retain efficacy without causing significant toxicity to globus pallidus or striatal neurons [51]. However, when tested in primates, treatment with AAV–TH–GCH did not result in any beneficial effect or functional improvement during a 6-month assessment period. Postmortem assessments of transgene expression demonstrated robust expression of *GCH*, but not *TH*, the cause of which remains unknown. These issues must be resolved before the AAV–TH–GCH vector can proceed to an early phase human clinical trial. In addition, it remains uncertain if the use of AAV provides an advantage over the current lentivirus-based triple gene therapy approach.

### SNCA Silencing

SNCA is a small, abundant protein that constitutes a major component of the intracellular Lewy bodies and neurites found in dopaminergic neurons in the substantia nigra of PD patients [52]. Studies of familial forms of PD have identified missense mutations in the gene encoding SNCA causing the development of an abnormal protein with abnormal function that in animals causes aggregation and parkinsonism. In addition to duplication and triplication of the locus containing the gene [53, 54], polymorphisms in its promoter [55] and other regions lead to increased expression [56]. The resulting overexpression leads to aggregation of SNCA that may lead to Lewy body formation and dopaminergic cell loss [57]. Gene therapies that could down-regulate SNCA might therefore have a role in the treatment of PD.

RNA interference (RNAi) is one method that could be used to reduce SNCA gene expression. RNAi utilizes sequences of RNA that are complimentary to specific regions of the messenger RNA of interest, blocking protein translation. Viral vectors can be used to deliver RNAi, and this method has been successfully implemented in preclinical studies to reduce the level of SNCA both in vitro [58, 59] and in vivo [60–62]. Nonetheless, several studies have suggested contradictory conclusions; though reduced SNCA expression is associated with a lower risk of developing PD [63], it is also associated with worse motor and cognitive outcomes [64]. Similarly, a preclinical study in a rat model reported motor deficits in addition to degeneration of nigral dopaminergic neurons with the knock-down of endogenous SNCA in the substantia nigra [65, 66]. These results raise the possibility that the relationship between SNCA expression and PD may be more complicated than is currently appreciated. Indeed, it

remains uncertain whether reducing SNCA would have a beneficial effect in PD. An alternate approach might involve inhibition of SNCA aggregation, and in vitro studies suggest that gene therapy with a mutant *SNCA* gene that blocks aggregation of wild-type PD-linked SNCA variants [67] could have a role in PD therapeutics.

### Parkin

Nearly half of all familial PD cases and 15 % of sporadic cases with onset before the age of 45 years are associated with mutations in the *Parkin* gene [68]. Parkin is a ligase that polyubiquitinates proteins destined for degradation by the proteasome [69]. The loss of Parkin function that results from mutations in familial PD can lead to the accumulation of potentially toxic substrate proteins in addition to dysfunctional mitochondria [70]. Thus, another approach to gene therapy in PD aims to restore normal Parkin function thereby protecting nigral neurons. In unilaterally treated 6-OHDA rats, transduction of nigral cells with a lentiviral vector encoding Parkin corrects motor asymmetry [71]. Likewise, in MPTP-treated mice, gene therapy with AAV–Parkin protects dopaminergic neurons [72]. Furthermore, in PD animal models in which SNCA is overexpressed, gene transfer of Parkin significantly reduces nigrostriatal degeneration resulting in behavioral improvements [73, 74]. Unfortunately, the single primate study conducted to this point has not reported neuroprotective effects, but it was limited by sample size and incomplete transduction [75]. Further preclinical work will be needed to determine if Parkin gene therapy may have a role in the treatment of PD.

### Other Approaches

RNAi could also be utilized for blocking other gene mutations associated with PD. For example, the PD-associated gene mutations in *leucine-rich repeat kinase 2 (LRRK2)* could be preferentially silenced over the wild-type *LRRK2*. A recent study reported effective targeting and subsequent silencing of 2 PD-related *LRRK2* mutations in vitro using RNAi [76], though whether this would reduce the risk of PD or affect the progression of PD remains uncertain. Overall, as gene therapy, including RNAi, can currently only target specific brain regions, attempting to alter expression of genes such as *LRRK2* and *SNCA* may be limited as expression of these genes is widespread. Nonetheless, targeting brain regions that are primarily involved in PD pathology may have some utility.

Mitochondrial dysfunction has been implicated in the pathogenesis of PD, and improving mitochondrial function could be a target of gene therapy. In vitro studies have suggested that replenishing mitochondrial DNA using a novel protein-mediated transfection technology (“ProtoFection”) may improve neuron function and prevent cell death [77], and it has

been suggested that mitochondrial gene therapy could have a role in sporadic PD treatment [78]. More specifically, replacement of the impaired mitochondrial nicotinamide adenine dinucleotide-quinone oxidoreductase (complex I) with a yeast alternative nicotinamide adenine dinucleotide dehydrogenase, Ndi1, has been shown to restore energetic balance and protect nigrostriatal dopamine cells both in vitro [77] and in vivo [79].

Neuroinflammation has also been implicated in the pathogenesis of PD, and gene therapy approaches to reducing neuroinflammation are also being studied. For example, in rat models of PD, both direct injection [80] and lentiviral delivery [81] of a gene for a dominant-negative tumor necrosis factor which selectively inhibits tumor necrosis factor, have been shown to reduce neuroinflammation and attenuate progressive loss of nigral dopamine neurons.

Though these gene therapy approaches for the treatment of PD remain theoretical and, in some cases, may either never enter or be years from human clinical trials, they demonstrate the wide variety of possible gene therapy targets for PD. As more is learned about the pathogenesis of PD, it is likely that new gene therapy targets will be identified.

### Conclusion

Over the last 10 years, several gene therapy approaches for the treatment of PD have entered human clinical trials. Some of these trials have produced promising results, while others have not, but the accumulating data suggest that gene therapy, targeting multiple brain regions including the striatum, STN, and substantia nigra, can be safe and well-tolerated in PD patients. Significant challenges remain, including how to modulate gene expression and how to determine the optimal target, dose, and patient population to study in future gene therapy trials. Advances in these areas may require the development of better animal models of PD, as well as improved methods for assessing efficacy (e.g., biomarkers). Nonetheless, novel gene therapy approaches to PD are currently being investigated in preclinical models, and advances in the understanding of the pathogenesis of PD and improvements in gene delivery methods will likely drive further development of gene therapies for PD.

**Required Author Forms** Disclosure forms provided by the authors are available with the online version of this article.

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