

# Gene Therapy for Parkinson's Disease

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**Abstract**— Today there are thousands of incurable diseases prevalent throughout the world and Parkinson's disease is one of them. It is a neurodegenerative disorder in which the patient's condition worsens over time. Medication therapy is used to cure symptoms of the disease and become less effective with time. Drugs such as levodopa or dopamine agonists are very effective during the initial stages of the disease but with time the medication response may fluctuate (wearing off). Hence these become less effective and may also have side effects. This is where gene therapy comes into play. Gene therapy has many potential advantages over medication therapy. It is a long term approach which slows down the disease progression. Gene therapy uses viral and non viral vectors to deliver the required nucleic acids to the target for their expression while maintaining vector biosafety (in case of viral vectors). The major viral vectors used are AAV and lentiviruses. Symptomatic therapy and disease modification are the two approaches under gene therapy in PD. Gene therapy based on the genetic cause of PD (recent) is a part of disease modifying approach which seems to have huge potential advantages as many genetic causes of PD are identified. In this paper the methods of gene therapy with respect to different approaches are summarized and recent clinical trials are also mentioned to show the current status of gene therapy in PD which will soon get us a complete cure of PD.

**Keywords** — Dopaminergic neuron, Gene-Therapy, Gene Delivery, Genetic Causes, Non viral therapy, Parkinsons disease, Viral techniques

## 1 INTRODUCTION

THE Parkinson's disease is a progressive, long lasting and multicentric neurodegenerative disorder of the central nervous system caused by death of dopaminergic neurons in the substantia nigra, region of mid brain. It leads to various motor symptoms like rigidity, shaking, bradykinesia etc. (Raymond T Bartus et al. 2013)

Currently, gene therapy is finding novel applications in the treatment of this disease because:

1. Pharmacological treatments do not give adequate and long term results and also have side effects.
2. As the cause of the neurodegeneration in parkinson's disease is well understood, it provides a specific target to the application of gene therapy. Gene therapy treats the disease by genetically modifying the populations of cells that are either directly impaired or capable of treating the disease symptoms. (Philippe G. Coune et al. 2012)

Gene therapy of Parkinson's disease includes:

1. symptomatic therapies / approaches
2. disease modifying approaches

The symptomatic therapies aim at suppressing the symptoms of the disease but do not slow down disease progression. In other words it is a short term approach whereas disease modifying approach provides long term relief by halting the disease progression (neuro protective function). This makes the disease modifying approach a favorable one. (Philippe G. Coune et al. 2012)

## 2 REVIEW OF LITERATURE

### 2.1 ABOUT THE DISEASE

Parkinson's disease is named after James Parkinson who first described this disease in a comprehensive way in

1817 (Sherwood et al. 1817).

### 2.2 SYMPTOMS

In PD at least two of the three cardinal signs bradykinesia, rigidity and tremor need to be present (Levy & Cummings, 1999). Other symptoms include flexed posture, "freezing," and loss of postural reflexes. (Stanley Fahn 2003).

Pathological symptoms include selective degeneration of the neuromelanin containing monoamine neurons, in the substantia nigra and the locus coeruleus (Stanley Fahn 2003). Within substantia nigra, ventrolateral part is usually most severely affected and this projects to putamen (Gibb WRG, Lees AJ 1991). Presence of lewy bodies (intracytoplasmic eosinophilic inclusions) is another main pathological symptom of this disease (mizuno et al. 1995).

### 2.3 CAUSES

The causes of Parkinson's disease are still unknown. Experts think that these are a combination of genetic and environmental factors (Parkinson's disease Foundation). During the last few years, possible genetic influences have become the focus of attention.

#### GENETIC FACTORS

In a minority of cases it occurs in a familial context. Mutation of the alpha-synuclein gene (chromosome 4q21-23) has been identified in one large Italian family and in five Greek families. That mutation gave rise to an autosomal dominant pattern of inheritance of parkinsonism. The clinical expression of the disease, however, was not quite typical for PD in the case of the Ala53Thr point mutation of the chromosome. For instance, the disease manifested itself at early ages. The Ala30Pro point mutation, recently discovered in a German family, however, resulted in a syndrome which phenotypically was much more like that seen in idiopathic sporadic PD (Kruger et al., 2001). Mutation of the Parkin gene (chromosome 6q25-27) has been identified as the cause of an autosomal-recessive juvenile form of Parkinsonism, although the start

of the disease at an early age and other atypical neuropsychological features again distinguish this condition somewhat from the 'common' form of PD. In the meantime, a spectrum of different alterations of the chromosome (various deletions or point mutations and others) have been found in the reported families. Thus, apparently a widely varying number of genetic pathological changes of several chromosomes can give rise to Parkinsonism (Klein et al., 2000).

### ENVIRONMENTAL FACTORS

Some researchers have suggested that exposure to an environment injury may result on Parkinson's disease. Several factors such as rural living, well water, manganese and pesticides have also been linked to Parkinson's disease through epidemiological researches.

Prolonged occupational exposure to certain chemicals is also found to be associated with an elevated risk of Parkinson's. These chemicals include insecticides beta-hexachlorocyclohexane (beta-HCH) and permethrin, the herbicides 2, 4-dichlorophenoxyacetic acid and paraquat and the fungicide maneb. In 2009, the US Department of Veterans Affairs added PD to a list of diseases possibly associated with exposure to Agent Orange. (Parkinson's Disease foundation, inc.)

A synthetic neurotoxin agent namely MPTP is found to cause immediate and permanent PD. This compound was discovered in the 1980s as it was found in individuals who injected themselves with a synthetic form of heroin which was contaminated with this neurotoxin. Cases of MPTP-induced PD are found to be very rare.

We do not have any effective evidence until now to prove that environmental factors can alone be the cause of Parkinson's disease.

## 3 GENE THERAPY IN PARKINSON'S DISEASE

The use of gene therapy to treat PD requires the use of a suitable method of delivery for the synthesized nucleic acid (viral or nonviral). The technique used for delivery is greatly influenced by the choice of vector as a peripherally administered vector must be able to cross the blood-brain barrier and that too with an acceptable degree of tissue specificity. Alternatively, to deliver the vector directly to a specific brain region, surgical techniques used for deep brain stimulation can be harnessed (Rachel Denyer et al. 2012).

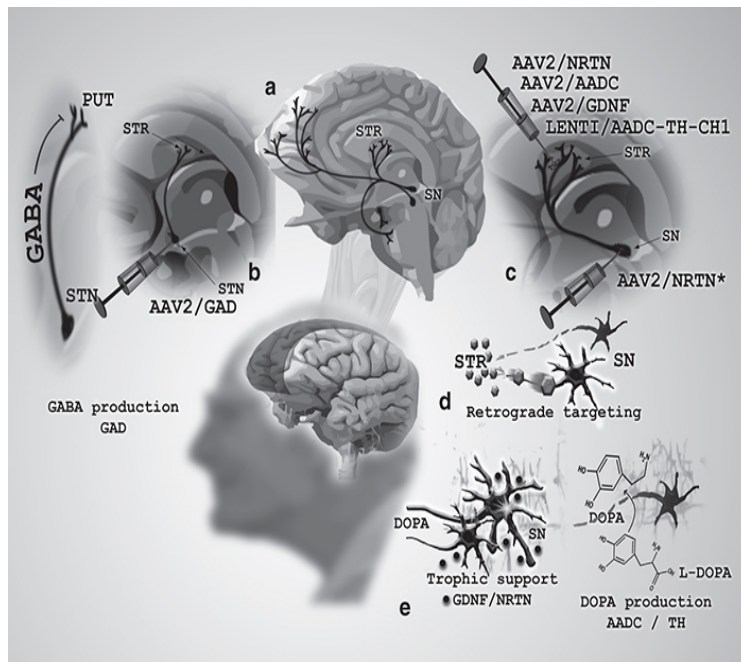


Figure 1. Gene therapy to date (2015)

### 3.1 NONVIRAL TECHNIQUES:

These are mainly used for transferring gene to the central nervous system (CNS).

It basically comprises chemical and physical methods like gene gun or electroporation. These techniques give a short duration of gene expression and hence are less suited for PD - a chronic neurodegenerative disorder (Philippe G. Coune et al. 2012). Low transfection rate is yet another drawback of using non viral techniques for translation to human studies (R. Huang et al. 2009). However this approach may still prove effective, as seen in the recent studies of GDNF (human glial cell - derived neurotrophic factor) gene and a neurotensin polyplex nanoparticle vector in an animal model of PD which induced a sufficient biochemical and functional response with a single intracerebral injection (J. A. Gonzalez -Barrios et al. 2006). Another nonviral vectors studied in animal models of PD have been incorporated with region-specific ligands to maximize the tissue specificity using intravenous vector administration (R. Huang et al. 2009).

### 3.2 VIRAL TECHNIQUES:

Viral vectors are obtained by removing the genes essential for replication from the wild-type virus's genome. The removed genetic information is provided in Trans for vector production, and is not incorporated inside the particles. Therefore the vectors are able to infect cells and transfer their genetic material into the nucleus, but are unable to replicate themselves in the host cells. This is very important to maintain vector biosafety (Philippe G. Coune et al. 2012).

These vectors derived from either DNA or RNA viral vectors, are considered to be a more practical approach than the non-

viral one, with the potential to cause long lasting gene expression via episome formation or DNA integration into the host genome. A range of different types of viruses, each with different properties and advantages, have been exploited in the search for a suitable vector for gene therapy in PD (Rachel Denyer et al. 2012).

Various types of vectors have been developed, differing by their tropism, packaging capacity, and immunogenicity. Today, in vivo gene transfer using viral vectors is the most commonly used approach in the CNS, with 20 trials listed in 2010 (Lim et al. 2010).

### 3.2.1 VIRAL VECTORS GENERALLY USED:

#### AAV ( Adeno-associated viruses )

Vectors derived from AAV (4.7 Kb) are most frequently used in clinical trials for CNS diseases. These are small nonenveloped viruses of the *parvoviridae* family. They comprise two genes encoding capsid (*cap*) and viral replication (*rep*) proteins and inverted terminal repeat sequences (Rachel denyer et al. 2012). The wild-type form of AAVs depends on the coinfection with helper virus (example- adenovirus or herpes virus) for efficiently replicating inside the host cells. AAVs are nonpathogenic in humans as their proteins (capsid) induce only mild immune reactions (Büning et. al. 2008); they are favorable candidates for gene therapy. However, according to a research about 90% of the population have been exposed to AAV serotype 2, and so have pre-existing antibodies that will possibly neutralize AAV2-based vectors and hence reduce their transduction efficacy (Moskalenko et. al. 2000). Genetic modifications in the capsid proteins can interrupt the immunogenic epitopes and possibly overcome this limitation (Maersch et al. 2010).

Viral vectors have are being derived from wild-type of AAVs by the deletion of all of the viral sequences except the ITRs so that AAV vectors cannot replicate and express any viral protein. When AAV vectors are produced, *rep* and *cap* genes are typically provided *in trans* using helper plasmid (system) cotransfected with the AAV transgene plasmid in presence of an adenovirus (Samulski et al. 1989). However, recently "helper-virus-free" systems have been developed, in which the helper activity is provided via the recombinant helper- plasmid (Grimm et al. 2003). After transfection, recombinant AAV particles are harvested by lysis of the packaging cells, purified, and finally concentrated in suspensions that typically contain  $10^{12}$ - $10^{14}$  particles per ml.

AAV vector particles can efficiently infect a broad range of cells, including postmitotic cells, and display a good neuronal tropism (Paterna et al. 2004). They enter the host cell through receptor-mediated endocytosis, and are translocated to the nucleus where their genome is converted into double-stranded DNA. Although wild-type AAVs preferentially integrate to the human genome at a specific locus on chromosome 19, termed AAVS1 (Samulski et al. 1991; Huser et al. 2010), recombinant AAV vectors form stable episomal concatemers in the absence of the Rep protein. In postmitotic cells, episomal

AAV genomes provide long-term (>1 yr) transgene expression. The low rate of integration of recombinant AAV vectors is considered an asset in their safety profile, as it limits the occurrence of insertional mutagenesis.

Overall, AAV vectors represent ideal vectors to deliver genes in the CNS. They present a very good safety profile, and provide efficient transduction and durable expression of neurons. The main limitation of AAV is the limited packaging capacity (4.7 kb) that precludes the integration of large genes, or multiple expression cassettes

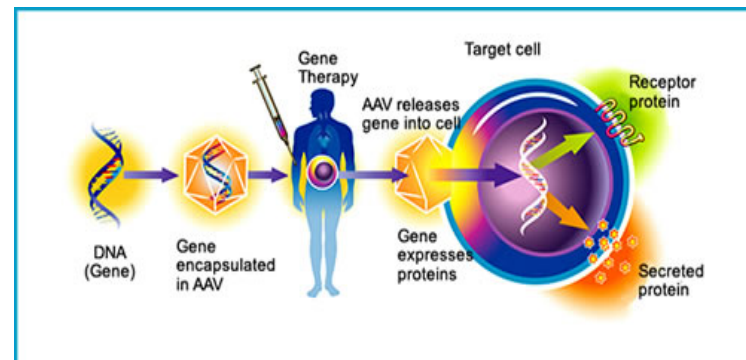


Figure 2. Gene therapy by AAV vector

#### LENTIVIRUSES

Unlike AAV, which has a limited transgene size (~4.7 kb), lentivirus vectors can accommodate a larger load (~8 kb) and are the current vector of choice for multigene PD treatments. Lentiviruses are RNA retroviruses capable of chromosomal integration and stable long-term expression. The most widely recognized wild-type lentivirus is the human immunodeficiency virus type I (HIV), the causative agent in acquired immune deficiency syndrome (AIDS). Because of the association of HIV with AIDS the use of lentivirus vectors for gene therapy has raised safety concerns. In order to increase safety, recombinant non-replicating and self-inactivating lentiviruses have been engineered that display tropism for neurons, transduce with high efficiency, and display stable long-term transgene expression. One non-human primate lentiviral vector system based on equine infectious anemia virus (EIAV) with the added capability of self-inactivation has been developed and is presently being used in PD clinical trials (Feng et al 2011).

HSVs AND ADENO VIRUSES ARE ANOTHER IMPORTANT VIRAL VECTORS USED.

### 4 SYMPTOMATIC THERAPY

Current pharmacological and surgical helps for PD all go for making up for the basal ganglia dysfunction brought on by the degeneration of the dopaminergic neurons from the substantia nigra pars compacta (Snpc). L-dopa (levodopa) helps in increasing the dopamine production in the remaining nigral neurons and in this way makes up for the absence of neurotransmitter in the striatum. Then again, deep brain

cerebrum electrical stimulation (DBS) regulates the overactivity of the subthalamic nucleus sequential to the loss of dopamine signaling in the striatum. Albeit both methodologies give symptomatic help to PD patients, they likewise introduce various downsides. Gene therapy methodologies have been explored for their capacity to comparatively right motor symptoms and conceivably lessen the event of unfavorable impacts connected with the current symptomatic medications (Philippe G. Coune et al. 2012).

#### 4.1 ENZYME REPLACEMENT STRATEGIES

Like L-dopa (levodopa) therapy, enzyme replacement therapies aim at compensating for the decrease of dopamine release resulting from the degeneration of the nigrostriatal dopaminergic neurons, by inducing ectopic dopamine synthesis. These approaches are based on the transfer of genes encoding the enzymes required for dopamine synthesis into the striatal GABAergic neurons. Endowed with these enzymes, the GABAergic neurons are able to ectopically synthesize dopamine that will be released in the striatum.

Although L-dopa therapy has been the mainstay of PD therapy since 1969 (Cotzias et al. 1969), its efficacy is limited by major side effects. Because dopaminergic drugs diffuse poorly into the CNS, they need to be systemically administered at high dose with an increased risk of peripheral adverse effects. At advanced stages of the disease, discontinuous L-dopa therapy is associated with the apparition of debilitating dyskinesia presumably linked to fluctuations in the brain concentration of L-dopa. Gene delivery of the enzymes needed for dopamine synthesis could provide a continuous ectopic production of dopamine in the striatum, which may reduce the apparition of dyskinesia and limit the side effects caused by elevated dopamine levels outside the basal ganglia.

#### Full Ectopic Dopamine Synthesis

A possible approach is to deliver the genes TH, AADC, and GCH1 to medium spiny neurons (MSN) in the striatum, to induce ectopic dopamine synthesis from tyrosine. A tricistronic EIAV-based lentivector coding for AADC and GCH1 and a truncated form of TH, has been developed by Oxford BioMedica under the name of ProSavin and successfully tested in 6-OHDA-lesioned rats (Azzouz et al. 2002). A study in MPTP-treated macaque monkeys (Jarraya et al. 2009) showed that ProSavin treatment does not only reduce motor impairment, but also leads to a drastic reduction of dyskinesia when compared to conventional L-dopa-therapy. The safety, efficacy, and dose evaluation of ProSavin are currently under evaluation in a phase I/II clinical trial.

#### Ectopic L-dopa Conversion

Genetic enzyme replacement therapy can also be used to increase the efficacy of pharmacological L-dopa therapy. The degeneration of the nigral dopaminergic neurons leads to a decrease in striatal AADC activity, which is essential for L-dopa conversion into dopamine (Ichinose et al. 1994). Therefore, the efficacy of the L-dopa treatment can be eventually limited by the loss of AADC activity. Advanced PD patients could benefit from gene transfer to restore striatal AADC levels, thereby lowering the effective dose of L-dopa and limiting the side effects. AAV vectors have been designed to deliver the AADC coding sequence to the striatal MSNs, which are then able to convert the administered L-dopa into dopamine. Striatal injection of AADC-coding AAV vectors was shown to effectively and stably restore the response to L-dopa in MPTP-treated rhesus macaque monkeys (Bankiewicz et al. 2000, 2006).

#### Ectopic L-dopa Synthesis

An alternative gene therapy approach aims at ectopically producing L-dopa in the striatum by transfer of the TH and GCH1 genes into MSNs. Endogenous AADC activity can convert the L-dopa produced in the striatum into dopamine. This strategy has been shown to improve motor deficits in rats presenting partial (Kirik et al. 2002) and total 6-OHDA-induced nigrostriatal denervation (Björklund et al. 2010), and to reduce dyskinesia caused by intermittent administrations of L-dopa (Carlsson et al. 2005). Interestingly, <sup>11</sup>C-raclopride positron emission tomography (PET) analyses indicates that functional recovery is correlated with the reconstitution of a functional pool of endogenous dopamine in the striatum (Leriche et al. 2009). This finding is surprising considering that there is no evidence that dopamine storage and release mechanisms are restored. Studies in nonhuman primates are currently ongoing to further assess the safety and efficacy of this gene therapy strategy

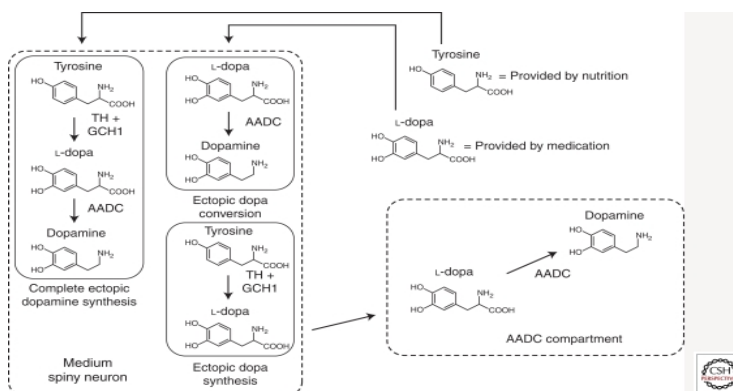


Figure 3. enzyme replacement therapy

## Glutamic Acid Decarboxylase Expression in the Subthalamic Nucleus

In 2002, Luo et al. described a gene therapy approach aiming at modulating subthalamic nucleus activity (Luo et al. 2002) by delivering the genes glutamic acid decarboxylase (GAD), the rate-limiting enzyme for the synthesis of GABA. Using an AAV vector, the approach targets the glutamatergic neurons of the STN. The neurons transduced with GAD synthesize and release GABA in an activity-dependent manner. The modified neurotransmitter pattern in STN projections leads to an inhibitory bias in the substantia nigra pars reticulata (SNr) and regulates the firing rates of basal ganglia nuclei in 6-OHDA-lesioned rats (Lee et al. 2005). These effects on the biochemical and electrophysiological properties of the STN-to-SNr projections improve the 6-OHDA-induced motor deficits in rats as a function of GAD expression levels (Luo et al. 2002; Lee et al. 2005). Many trials have confirmed the safety of the GAD gene transfer approach in the STN, and reported beneficial clinical effects. However, the improvements obtained have been so far modest—therefore, this approach should be compared with DBS in terms of safety and efficacy before any large-scale application could be envisioned. In conclusion, the exploration of symptomatic gene therapies for PD has provided encouraging, preclinical and clinical results and has showed that current viral vectors technology allow for safe gene delivery within the basal ganglia nuclei.

During the last decade, the discovery of genes linked to familial forms of PD has dramatically improved our understanding of the possible molecular causes of neurodegeneration.

Based on these findings, novel approaches have been proposed to possibly protect neuronal functions and halt disease progression. In this context, gene therapy offers an attractive alternative to deliver genetic information in the CNS and chronically provide the needed neuroprotective effects. Here we will discuss the use of neurotrophic factors to support the function and survival of nigral dopaminergic neurons, as well as therapeutic approaches to correct the genetic defects associated with familial forms of PD.

### Neurotrophic Factors: GDNF

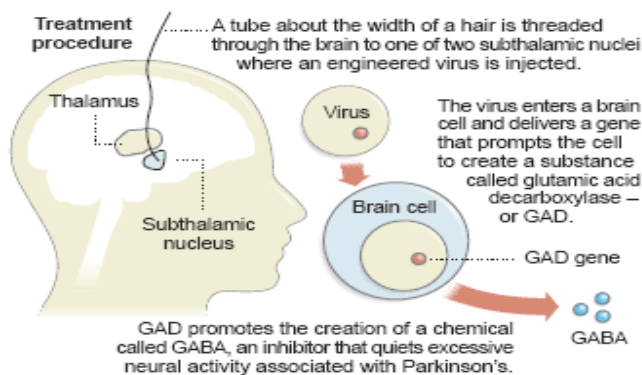
Neurotrophic factors are secreted proteins playing essential roles in the differentiation, growth, and survival of neuronal cells. In particular, the glial cell line-derived neurotrophic factor (GDNF), a member of the transforming growth factor (TGF)- $\beta$  superfamily, has emerged as a powerful factor to protect the dopaminergic neuronal function and has therefore been intensely investigated as a promising target for PD therapy. GDNF was identified in 1993 for its potent trophic activity on dopaminergic neurons in vitro (Lin et al. 1993). Since then, GDNF has been shown to promote axonal sprouting in vivo (Beck et al. 1995) and to be essential for the survival of dopaminergic neurons in the adult brain (Pascual et al. 2008).

### Direct GDNF Injections

As the recombinant protein GDNF does not cross the human blood-brain barrier (Deierborg et al. 2008), it is crucial to find alternatives to systemic administration for PD treatment. The local delivery of recombinant GDNF protein into CNS has been investigated. Direct injections, or infusion of GDNF using minipumps into the striatum, midbrain, or ventricles provided encouraging results in toxin-based rodent and primate models of PD (Kearns and Gash 1995; Sauer et al. 1995; Grondin et al. 2002,2003; Ai et al. 2003). Notably, it has been found that GDNF can induce the sprouting of dopaminergic axons near the site of GDNF delivery (Rosenblad et al. 1999; Kirik et al. 2000). Based on the preclinical results, a randomized double-blind clinical study was initiated using intracerebroventricular GDNF infusion (Nutt et al. 2003). The results were disappointing, with no clinical improvement and adverse effects, including anorexia. The postmortem analysis of one of the participant confirmed the absence of neuroprotection and revealed

### **New step in Parkinson's treatment**

Preliminary evidence suggests that an experimental gene therapy procedure could ease Parkinson's symptoms with no side effects.



SOURCE: NewYork-Presbyterian Hospital-Weill Cornell Medical Center AP

## **5 DISEASE MODIFYING APPROACH**

The main therapies available for the treatment of PD aim at restoring dopamine levels or correcting the functional perturbations of the basal ganglia caused by dopamine loss. However, these symptomatic therapies do not slow down disease progression, and their efficacy therefore declines over time.

the absence of GDNF diffusion into the brain parenchyma (Kordower et al. 1999). Therefore, it appeared essential to change the mode of GDNF delivery. Indeed, a subsequent pilot study using striatal infusion with a minipump system reported positive clinical results (Gill et al. 2003). However, a double-blind phase II clinical trial run by Amgen, failed to confirm these results, and the company decided to abandon further clinical investigation of direction GDNF infusion in the CNS. Nevertheless, these trials provided a strong rationale for gene therapy as a potential alternative to the intraparenchymal delivery of recombinant neurotrophic factors.

### In Vivo GDNF Gene Delivery

In contrast to the delivery of the recombinant protein, gene transfer allows for a constant and local administration of GDNF, which limits the risks of side effects associated with broad distribution of this factor, such as loss of weight and allodynia (Hoane et al. 1999). As genetically modified cells continuously synthesize GDNF, this approach would avoid protein stability issues and the potential need for repetitive interventions to inject recombinant GDNF or reload implanted minipumps. GDNF gene delivery has been successfully evaluated in aged monkeys that displays naturally occurring mild reduction of striatal dopamine and motor deficits (Kordower et al. 2000; Palfi et al. 2002; Johnston et al. 2009). Surprisingly, GDNF fails to provide any neuroprotective effect or induce striatal axonal sprouting in nigral neurons overexpressing  $\alpha$ -synuclein for reasons that remain to be elucidated. Although the effect of GDNF gene therapy is still investigated preclinically, two clinical trials have been conducted with an AAV2 vector expressing the neurotrophic factor neurturin, a close homolog of GDNF. Preclinical studies showed that intrastriatal injections of the AAV2-neurturin vector protect nigral neurons from 6-OHDA-induced degeneration and preserves animal motor behavior (Gasmi et al. 2007a,b).

Using viral vectors for the delivery of neurotrophic factors, there is a distinct risk that viral particles could diffuse away from the site of injection and elicit ectopic GDNF expression, which unpredictable consequences. Because of these safety concerns, alternative systems for ex vivo gene transfer have been explored. The implantation of genetically engineered stem cells (Akerud et al. 2001; Park 2001) or astrocytes (Cunningham and Su 2002) has been proposed. To further improve the safety of the approach and protect the grafted cells from potential host immune reactions, it is possible to implant cells genetically engineered for GDNF expression within a permeable polymer capsule (Tseng et al. 1997; Kishima et al. 2004; Sajadi et al. 2006). However, the efficacy of these approaches has not been investigated in PD patients yet.

In conclusion, the potential regenerative and neuroprotective effects of neurotrophic factors justify further investigation in PD. However, it is essential to evaluate the efficacy of this approach on the genetic factors of PD.

## **5.1 Gene Therapy Based on the Genetic Causes of PD**

In the last decade, several genes implicated either in the Mendelian inheritance of PD or as risk factors for sporadic PD have been identified. These findings have dramatically changed our understanding of the disease process and provide new clues for genetic strategies that aim at slowing down the degeneration of vulnerable populations of neurons, in both familial and sporadic forms of PD.

### **$\alpha$ -Synuclein**

The small protein  $\alpha$ -synuclein is considered as a major actor in both sporadic and familial cases of PD. Missense mutations in the  $\alpha$ -synuclein coding sequence were initially found in rare families with autosomal dominant PD. Interestingly, similar cases of PD were associated with polymorphisms in the  $\alpha$ -synuclein promoter (Maraganore et al. 2006), and multiplications of the locus carrying the  $\alpha$ -synuclein gene (Singleton et al. 2003; Nishioka et al. 2006). This last finding clearly links certain forms of PD with the expression level of the protein. One can therefore assume that strategies to down-regulate  $\alpha$ -synuclein expression may impact on the disease process. Several viral vector-based gene delivery systems have been explored to interfere with  $\alpha$ -synuclein expression. They mainly rely on RNA interference (RNAi) to selectively destabilize the  $\alpha$ -synuclein mRNA and/or block protein translation, via the transgenic expression of short hairpin RNAs (shRNA), or micro RNAs (miRNA) directed against the  $\alpha$ -synuclein mRNA sequence. An alternative approach based on the AAV-mediated delivery of an anti- $\alpha$ -synuclein ribozyme has also been investigated both in vitro and in vivo (Hayashita-Kinoh et al. 2006).

RNAi has been shown to successfully reduce the level of both endogenous or overexpressed  $\alpha$ -synuclein, either in vitro (Fountaine and Wade-Martins 2007; Junn et al. 2009) or in vivo (Sapru et al. 2006; McCormack et al. 2010). In vitro silencing of A53T  $\alpha$ -synuclein in NS20Y cells was found to decrease proteasome impairment caused by  $\alpha$ -synuclein and increase the cell resistance to oxidative stress (Junn et al. 2009) supporting potential protective effect of this approach.

However, a recent study reported that knock-down of endogenous  $\alpha$ -synuclein in the adult rat SN leads to the degeneration of nigral dopaminergic neurons and motor deficits (Gor-

batyuk et al. 2010). Although the mechanisms underlying this effect remain unclear, it appears that extent of neurodegeneration correlates with the degree of  $\alpha$ -synuclein silencing. Considering that the normal function of  $\alpha$ -synuclein is still poorly understood, the potential consequences of uncontrolled  $\alpha$ -synuclein silencing should be carefully addressed, as the near complete loss of  $\alpha$ -synuclein may have negative effects on neuronal function and survival. Although challenging, it may be important to devise strategies to genetically control the degree of  $\alpha$ -synuclein silencing to safely implement this approach in PD patients.

## Parkin

Parkin has been linked with PD by the discovery of mutations associated with autosomal recessive juvenile parkinsonism (AR-JP), an early onset form of PD with typical symptoms and pathology and very slow disease progression (Kitada et al. 1998). Parkin is an E3 ubiquitin ligase that polyubiquitylates proteins destined for degradation by the proteasome (Shimura et al. 2000). AR-JP mutations lead to partial or complete loss of the protein function (Shimura et al. 2000), and therefore to the accumulation of potentially toxic substrate proteins. In addition to protein ubiquitylation, there is also evidence for beneficial effects of Parkin expression on oxidative stress levels (Hyun et al. 2002), and more recently, on mitochondrial homeostasis, as illustrated by the Parkin-induced autophagic elimination of dysfunctional mitochondria (Narendra et al. 2008). Therefore,

Parkin appears as potential neuroprotective agent with a broad mode of action, which may contribute to neuronal resistance to various stressors potentially implicated in sporadic forms of the disease as well.

In rats with a partial unilateral 6-OHDA lesion, a lentiviral vector encoding Parkin had a significant neuroprotective effect, leading to a correction of motor asymmetry for 20 weeks (Vercammen et al. 2006). Using a more severe 6-OHDA-induced lesion, a subsequent study using an AAV vector did not confirm the neuronal protection, but reported a decrease in drug-induced rotametry and spontaneous behavior in conditions of Parkin overexpression (Manfredsson et al. 2007). It was suggested that Parkin could improve motor function via increased levels of tyrosine hydroxylase and striatal dopamine, thereby enhancing dopamine striatal neurotransmission. In MPTP-treated mice, an AAV vector for Parkin expression was reported to induce significant neuroprotection (Paterna et al. 2007).

Overall, viral vectors for Parkin expression have shown neuroprotective effects in various animal models of PD. Targeting

the subset of patients afflicted by AR-JP associated to Parkin deficiency appears as an obvious strategy for the clinical application of this etiologic gene therapy approach. However, despite an early onset, the Parkin-linked form of the disease has a very slow progression (Khan et al. 2002), with a good response to L-dopa therapy. These features clearly raise the bar for gene therapy application. It is therefore important to further explore the function of Parkin and perform more extensive preclinical studies in nonhuman primates, to determine the possible detrimental effects of long-term Parkin overexpression.

Symptomatic approaches	Transgenes	Viral vector	Preclinical studies			Clinical studies		Current status
			In vitro studies	Rodent models	Nonhuman primate	Phase I	Phase II	
Full ectopic dopamine synthesis	TH + GCH1 + AADC (Prosaavin)	EIAV	→	→	→	→	→	Motor response and reduced dyskinesia in rodent and primate models Phase III clinical trial ongoing
Ectopic L-dopa conversion	AADC	AAV2	→	→	→	→	→	Open Phase I clinical trial: modest clinical improvements in both on- and off-states
Ectopic L-dopa synthesis	TH + GCH1	AAV5	→	→	→	→	→	Motor response and reduced dyskinesia in rodent models, non-human primate studies ongoing
GAD expression in the STN	GAD65/67	AAV2	→	→	→	→	→	Phase II clinical trial: significant improvement of the UPDRS score (~8.1 off-medication)
Disease-modifying approaches								
Neurotrophic factors gene delivery	BDNF/Neurturin (CERE-120)	Lentivector/ AAV2	→	→	→	→	→	Phase II clinical trial: no significant improvement of the UPDRS score (AAV2-Neurturin)
$\alpha$ -Synuclein silencing	N/A	Lentivector/ AAV2/5	→	→	→	→	→	Suppression of $\alpha$ -synuclein with viral vectors and siRNA infusion Potential detrimental effects of extensive silencing
Parkin gene delivery	Parkin	Lentivector/ AAV2	→	→	→	→	→	Preclinical studies: neuroprotective effect in animal models

## 6 CONCLUSION

Impediments in the benefit of medicinal and the surgical medications of PD have animated deliberations to create new therapies. Gene therapy has different advantages over traditional treatment for PD as it may safeguard or restore dopaminergic neurons through the utilization of growth factors or then again expand the accessibility of proteins needed for dopamine production. In the course of recent years, 2 separate approaches have developed and have been executed in carefully designed human treatment conventions. To date, seem, by all accounts, there is some confirmation proposing profit. It is trusted, keeping in mind the ongoing clinical trials and their results that gene therapy will soon give enhanced treatment choices to individuals with Parkinson's disease.

## 7 Acknowledgments

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