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Review Article

# Genetic Abnormalities behind the Parkinson's Disease, and its Therapeutic Intervention

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## Abstract

Parkinson's Disease (PD) is the second highest neurodegenerative disease after Alzheimer's and is caused by a progressive loss of dopamine producing neurons in the Substantia Nigra compacta (SNc). The disease is characterized generally by muscular tremor, difficult postures and forgetfulness, and at the molecular level is recognized by the appearance of intraneuronal α-synuclein-aggregates. Therapies at the present time are mainly palliative like administration of Dopa/Dopmaine to replenish the loss or to supply from shortage of it, which is needed for normal movement of the body muscles and to maintain the postures. PD that develops at the early stage of life is linked with genetic abnormalities, however, till date it is described mainly as an old age, sporadic and idiopathic. The present review will focus on the molecular mechanisms of PD development as well as its therapeutic intervention.

**Keywords: Parkinson's disease; Alzheimer's; Genetic abnormalities; Intraneuronal α-synuclein-aggregates**

## Abbreviations

PD: Parkinson's Disease; SN: Substantia Nigra; L-Dopa: L-Dihydroxyphenylalanine; VTA: Ventral Tegmental Area; DA: Dopamnine; iPSCs: Induced Pluripotent Stem Cells; PARK1: Gene which Code for alpha-synuclein (SNCA); PINK1: PTEN-Induced Putative Kinase 1; DJ1: Protein Deglycase DJ-1, also known as Parkinson disease protein 7; *LRRK2*: Leucine-Rich Repeat Kinase 2; SNCA: Synuclein-α

## Introduction

PD is generally characterterised as a tremor problem with weak postures which are progressive. Other symptoms include bradykinesia, forgetfulness, anxiety, depression, and ultimately death may appears [1-6].

The pathological hallmark of the disease is the deficiencies of the Dopamine (DA) in the Substantia Nigra Compacta (SNc) region of the brain and the presence of Lewy bodies composed of aggregated α-synuclein, neurofilaments and ubiquitin in neuronal and glial cells across the neural cell transmission pathaways [7-9].

It has been calculated that symptoms of PD may develop in humans that lose 48% to 68% of the dopaminergic neurons at the SNc and/or loss of DA content around 70% to 80% at the striatum [10,11]. Although PD is generally known as an old-age disease, it can also be found at the early stage of life (14% to 16%), as early as age 7. Those cases are mainly associated with their inheridatory linkage or any mutations in their neural circuit proteins [12]. In this review article, we will focus on gene products related to PD genesis and strategies to develop its therapeutic approaches.

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## Molecular Pathogenesis

### Functional disorganization of the dopaminergic neurons in the midbrain

Dopaminergic neurons are located in the SNc and VTA (Ventral Tegmental Area). These neurons innervate the dorsal and lateral striatum and produces dopamine that's necessary for controlling the motor movements [13]. The key rate-limiting enzymes for biosynthetic pathway of dopamine are tyrosine hydroxylase, which hydroxylate tyrosine to L-3,4-hydroxyphenylalanine (L-Dopa) and Amino Acid Decarboxylase (AADC) which converts L-dopa to dopamine.

As the disease progresses, there are consistent elimination of nigral neurons, which leads to a serious loss of dopamine in the brain. This loss warrants an increased administration of dopamine or its precursor L-dopa from outside to generate a clinical effect. However, an excessive stimulation may cause hallucinations and motor neuron defect in them.

### Mitochondrial dysfunction

The most complex subcellular organelles of eukaryotic cells are mitochondria, which primarily produce ATP by oxidative phosphorylation and support the metabolic pathways of Krebs cycle. Mitochondria remove toxic free radicals and regulate neuron apoptosis and maintain calcium homeostasis [14,15]. Therefore, any mitochondrial dysfunction may cause oxidative stress and neuroinflammation in cells. Previous studies have shown that during aging process the absence of antioxidant defense mechanisms cause the vulnerability of mitochondrial DNA (mtDNA) in humans [14,16-18]. Furthermore, telomere removal can enhance α-synuclein protein aggregation, a mechanism of loosing neural circuit and development of PD [19]. A recent study with CRISP9/Cas9-mediated telomere removal from neuroblastoma cells (SHSY5) showed mitochondrial dysfunction and cell death [19]. This evidence suggests that the prevention of mitochondrial dysfunction could be an effective therapeutic approach of PD.

### Involvement of genetic defects

Despite of low heredity-related cases of PD (14% to 15% only),

some rare familial forms indicate the genetic abnormalities cause PD pathology. Currently, 28 chromosomal regions have been found to be linked with PD cases; and six of them contain genes such as α-synuclein (SNCA /PARK1), *LRRK2* (PARK8), PINK1 (PARK6), Parkin (PARK2), DJ-1 (PARK7), *ATP13A2* (PARK9), which when mutated causes PD (3% to 5% of total cases) [12,18]. The remaining cases of PD still considered as idiopathic [12].

**α-Synuclein:** SNCA makes the protein α-synuclein. In brain cells of individuals with Parkinson's disease, this protein gathers in clumps called Lewy bodies. Mutations in the SNCA gene occur in early-onset Parkinson's disease.

The small protein α-synuclein is considered as a major factor for both sporadic and familial cases of PD. Missense mutations in the α-synuclein coding sequence were found with autosomal dominant PD. The mutated α-synuclein can interact with anionic lipids and results aggregation into toxic complexes. They interfere microtubule transport [14,20], produces reactive oxygen species and ultimately promotes neuronal death [21].

***Parkin* (PARK2):** An early onset form of PD with autosomal recessive juvenile parkinsonism has been linked with mutations in *Parkin* gene [22]. Parkin is an E3 ubiquitin ligase that destined for degradation of any cell-destructive proteins, therefore, mutation of Parkin lead to partial or complete loss of the ubiquitination function and causes the cell death from toxic substrate proteins [23,24].

In rats with a partial unilateral 6-OHDA lesion, a lentiviral vector encoding Parkin had a significant neuroprotective effect [25]. 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 [26]. Studies conducted in rats using lentiviral [27] and AAV [28] vectors showed that Parkin overexpression significantly reduces the α-synuclein-induced nigrostriatal degeneration, and leads to behavioral recovery [28]. However, the only primate study conducted yet did not report a protective effect of Parkin against the α-synuclein-induced loss of dopaminergic neurons in the SNpc [29].

***PINK1* (PARK6):** Mutations in the Phosphatase and Tensin Homolog (PTEN)-induced putative kinase 1 (*PINK1*) gene are the second most common cause of AR EOPD (autosomal recessive-early onset PD). The frequency of *PINK1* mutations is in the range of 1% to 9%, with considerable variation across different ethnic groups [30-35].

*PINK1* is a 581 amino acid ubiquitously expressed protein kinase. It consists of an amino-terminal 34 amino acid mitochondrial targeting motif, a conserved serine–threonine kinase domain (amino acids 156-509; exons 2-8), and a carboxy-terminal autoregulatory domain. Two-thirds of the reported mutations in *PINK1* are loss-of-function mutations affecting the kinase domain, demonstrating the importance of *PINK1*'s enzymatic activity in the pathogenesis of PD. Interestingly, recent studies provided evidence that *PINK1* and Parkin function in a common pathway for sensing and selectively eliminating damaged mitochondria from the mitochondrial network. *PINK1* is stabilized on mitochondria with lower membrane potential, and as such, it recruits Parkin from the cytosol. Once recruited to mitochondria, Parkin becomes enzymatically active and initiates the autophagic clearance of mitochondria by lysosomes, i.e., mitophagy [36].

Interestingly, in contrast to Parkin, the majority of PINK1 mutations reported are either missense or nonsense mutations, and, to date only three families with whole-exon deletions (exons 4-8 [37], 6-8 [38], and 7 [39], and one with a heterozygous whole-gene deletion have been reported [40]. More than 60 different missense and nonsense mutations were found in >170 patients, affecting all 8 *PINK1* exons at nearly equal frequencies (in each of the exons 5–10 different mutations were reported).

**DJ-1 (PARK7):** DJ-1 is the third gene associated with AR PD, and it is mutated in about 1% to 2% of EOPD (Early Onset PD) cases [41]. Given that DJ-1-linked PD seems to be rare, very few patients have been reported in the literature.

The seven coding exons of the DJ-1 gene code for a 189-amino acid-long protein that is ubiquitously expressed and functions as a cellular sensor of oxidative stress [42,43]. The DJ-1 protein forms a dimeric structure under physiologic conditions [44], and it seems that most of the disease-causing mutants (p.L166P, p.E64D, p.M26I, and p.D149A) heterodimerize with wild-type DJ-1 [45]. In addition, the mutated proteins are frequently not properly folded, unstable, and promptly degraded by the proteasome. Thus, their neuroprotective function and antioxidant activity are reduced [46,47].

***LRRK2* (PARK8):** *LRRK2* is a large gene that consists of 51 exons. It encodes the 2527-amino acid cytoplasmic protein Leucine-Rich Repeat Kinase 2 (*LRRK2*) that consists of a leucine-rich repeat toward the amino terminus of the protein and a kinase domain toward the carboxyl terminus with various conserved domains in between. There are more than 50 different missense and nonsense mutations reported in *LRRK2* to date [48], and at least 16 of them (including the six recurrent mutations-p.R114C, p.R1441G, p.R1441H, p.Y1699C, p.G2019S, and p.I2020T) seem to be pathogenic.

Mutations in the *LRRK2* gene are the most frequent known cause of late-onset autosomal-dominant and sporadic PD, with a mutation frequency ranging from 2% to 40% in different populations [49-51]. Patients respond favorably to levodopa therapy, and dementia is not common. Neuropathological findings are mostly inconsistent, showing both Lewy body (and sometimes tau- and ubiquitin-containing inclusions) pathology and pure nigral degeneration without Lewy bodies, with or without neurofibrillary tangles [52].

The pathogenic mechanism leading to PD caused by *LRRK2* mutations is still uncertain. *LRRK2* is a large protein with many domains capable of protein-protein interactions, and thus it is plausible that changes in these domains would influence the *LRRK2*'s relationship with other proteins, i.e., currently unknown interactors with which it forms complexes or which it phosphorylates.

***ATP13A2* (PARK9):** *ATP13A2* is a large gene comprised of 29 exons coding for an 1180-amino acid protein. The *ATP13A2* protein is normally located in the lysososmal membrane and it has 10 transmembrane domains and an ATPase domain [53]. Homozygous and compound-heterozygous mutations in *ATP13A2* have been found to cause an atypical form of PD named Kufor-Rakeb syndrome [53]. This syndrome has juvenile onset with rapid disease progression, accompanied by dementia, supranuclear gaze palsy, and pyramidal signs.

**Other genes with possible role in PD:** Apart from the genes causing the six monogenic forms of PD, changes in a large number of additional genes were considered PD-causative and identified by

linkage analysis or a candidate gene approach. Some of these genes even attained a "*PARKI*" designation (*UCHL1* [*PARK5*], *GYGYF2* [*PARK11*], OMI/*HTRA2* [*PARK13*], *PLA2G6* [*PARK14*], and *FBXO7* [*PARK15*]). However, as discussed in the Genetic Classification of PD section, the link of some of these genes to PD is uncertain and not confirmed.

## Gene Therapy: Strategic Intervention

### Strategies to down-regulate α-synuclein

Several viral vector-based gene delivery systems have been explored to interfere with α-synuclein expression. RNA interference (RNAi) to selectively destabilize the α-synuclein mRNA and/or block protein translation, via the transgenic expression of short hairpin RNAs (shRNA), or micro RNAs (miRNA) directed against the α-synuclein mRNA sequence, was explored with success. RNAi has been shown to successfully reduce the level of both endogenous or over expressed α-synuclein, either *in vitro* [54,55] or *in vivo* [56,57].

*In vitro* silencing of A53T α-synuclein in NS20Y cells was found to decrease proteasome impairment caused by α-synuclein and increase the cell resistance to oxidative stress [55]. However, a recent study reported that knock down of endogenous a-synuclein in the adult rat SN leads to the degeneration of nigral dopaminergic neurons and motor deficits [58]. Although the mechanisms underlying this effect remain unclear, it appears that extent of neuro-degeneration correlates with the degree of α-synuclein silencing. Complete loss of a 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 α-synuclein silencing to safely implement this approach in PD patients.

### Parkin expression has further proved neuroprotective in genetic animal models of PD

In Drosophila, Parkin expression has been shown to reduce dopaminergic neurons degeneration induced by the Parkin substrate PaelR [59]. Studies conducted in rats using lentiviral [27], and AAV [28] vectors showed that Parkin over expression significantly reduces the α-synuclein-induced nigrostriatal degeneration, and leads to behavioral recovery [28]. The only primate study conducted yet did not report a protective effect of Parkin against the a-synuclein-induced loss of dopaminergic neurons in the SNpc [29].

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

### A potential gene therapy using AADC

AADC is the enzyme which converts dopa to Dopamine. Striatal injection of rAAV-AADC alone in a rat model of Parkinson's disease increased behavioral responsiveness following L-Dopa administration [60]. These results supported the establishment of a phase I clinical trial involving gene transfer of AADC, and that is currently underway.

### Protection of neural cells by GDNF gene

In rodent and primate models of Parkinson's disease has shown that intranigral, intrastriatal, or intraventricular injection of GDNF has both protective and restorative properties. Indeed, GDNF protects dopaminergic neurons and restores behavioral deficits, induced by the toxins 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 6-hydroxydopamine (6-OHDA) [61-63].

### The cholinergic system

It plays a broad role in controlling neurotransmitter release, reducing neuroinflammation, and promoting neuronal survival and synaptic plasticity in the brain. The binding of Acetylcholine (ACh) to nicotinic Ach receptors (nAChRs) occurs throughout the brain, including within striatum and other constituents of the mesolimbic, mesocortical, nigrostriatal, and frontostriatal loops. nAChRs are pentameric ligand-gated ion channels composed of a-subunits (α2-α7) or containing α and β-subunits (β2-β4) [64]. Presynaptic nAChRs mediate neurotransmitter release and postsynaptic receptors increase neuronal firing rates and thus facilitate long-term potentiation.

As discussed above, the striatum contains large a spiny Cholinergic Interneurons (ChIs) that interact with DA inputs. While ChIs is the primary source for ACh in the striatum, cholinergic projections also arrive from the Pedunculopontine Nucleus (PPN) and the laterodorsal tegmental nuclei [65]. DA depletion in the striatum causes increased the excitability of ChIs as a consequence of the loss of the inhibitory dopaminergic modulation via presynaptic D2-like receptors on ChIs. Deficits in ChI function is involved in various basal ganglia-related movement disorders such as dystonia, PD, and Tourette's syndrome [65-67]. Striatal ChIs appear to support synaptic plasticity and cognitive functions, mediated by the dorsal striatum such as attention, and motivation [67-71]. ChIs and DA work together to regulate motor function and represent good targets to alleviate PD symptoms [72].

In the striatum, ChIs express the muscarinic acetylcholine receptors (mAChRs; M1/M5) as well as various subtypes of nAChRs, composed mainly of α4, α6, α7 and β2, and β3 subunits, with the primary expression of the α4 β2 and α6 β2 receptors [73]. Striatal nAChRs are expressed in dopaminergic and glutamatergic neurons, as well as ChIs and GABA ergic interneurons [74,75]. On the other hand, nAChRs are absent from MSNs [76].

Several studies have investigated changes in the expression of muscarinic and nAChRs in PD [77-85]. A4 β2 and α7nAChRs were also found to be reduced in the cortical and subcortical regions of the brain, including the frontal and temporal cortices, hippocampus, caudate nucleus, and the pons of patients with PD when compared to healthy controls [86-88]. It has also been reported an inverse correlation between the level of dementia and nAChRs expression in the hippocampus and temporal cortex of the PD patients [89].

Other studies showed severe losses in α6 β2 receptor expression and a minor decline in the α4 β2 subtypes in PD brains. The decrease in α6β2 but not α7 receptors paralleled a reduction in markers of nigrostriatal degeneration [90]. Within the putamen, there was no change in the expression of the α2-α7, β2 and β3 nicotinic subunits and the authors suggested that the observed binding deficits may be the result of a change in the assembly of the receptors' subunits likely induced by a-synuclein instead of a change in the expression of the nicotinic subunits in the striatum [91].

Brain imaging studies using positron emission tomography revealed a decrease in the number of nAChRs in the amygdala of patients with PD [89,92-97]. This is similarly true in frontal and parietal cortices, the striatum, and substantia nigra in the PD brain [98].

## Delivery of Genes - a Next Concern for Effective Gene Therapy

### CRISPR technology

Methods of gene delivery, including viral vectors and CRISPR, have been developed [99]. Recently one of the latest advances in

CRISP technology was reported by [100] from Harvard University [101]. In this new approach, Cas9 hybridizes to the target DNA site using a guide engineered RNA containing a complementary spacer. To transfer the latest information from these guide RNAs, the genomic DNA is nicked at only one location [101]. This method reduces the risk of undesired DNA mutations, and it may very well revolutionize the therapy of PD and other pathologies linked to single-gene mutations.

### Viral vectors

Another promising approach lies in gene therapy using non-replicating viral vectors such as Adeno-Associated Virus (AAVs), retro and lentiviruses [102], and glycoprotein-deleted rabies virus [103-107]. Gene delivery using AAVs has the advantage in that these viruses do not integrate into host chromosomes yet persist as episomic chromosomes that do not provoke insertional mutations and permit stable gene expression in neuronal and glial cells [108]. Furthermore, AAVs do not induce immunoreactions in humans and, as a result, are regarded as one of the best viral gene delivery systems for use in preclinical biomedical research and clinical trials [109]. However, the main limitation of AAVs is that they can only deliver up to 5.2 kb of genetic material [110]. Lentiviruses, however, can deliver genetic sequences of up to 9 kb to dividing and non-dividing cells. After transduction, the lentiviral RNA is reverse transcribed to DNA and randomly integrated into the host chromosomes [111]. This disadvantage limits its clinical application though lentiviruses are frequently used in preclinical research [112].

The recent approval of human AAV vector use in Europe and the USA has led to an array of gene therapy attempts in various clinical trials [113-115]. The genetic approaches taken for PD treatment are largely neuro-regenerative in nature, and they are directed to halt neuronal cell death. For example, some strategies include inducing the over expression of neurotrophic factors in the substantia nigra or the increasing repair genes to disrupt the formation and accumulation of aggregated and neurotoxic forms of neuronal proteins such as α-synuclein. More than a decade ago, a pioneering phase1 study assessing the safety of Human Aromatic L-Amino Acid Decarboxylase (hAADC) gene therapy for PD tested the effect of bilateral AAV2-induced AADC expression in the putamen of subjects with advanced PD [116]. Although the authors reported no adverse effects of AAV-mediated AADC over expression in humans, they found no significant clinical recovery [116]. Follow-up clinical studies reported positive effects, such as reduction of symptoms as well as lowered L-DOPA dosage required for treatment.

## Conclusions

The complexity of PD genesis including the heterogeneity of unigene or multi genes defect for neural cells loss makes it difficult to develop a preventive or curative therapies for PD. Further, the absence of reliable biomarkers to diagnose PD at an early stages, and also the lacking of an appropriate animal model makes it challenging in development of PD therapies.

Gene therapy offers a promising potential treatment avenue for PD with the theoretical possibility of targeting both non-disease and disease modifying targets. While encouraging results have been obtained in clinical trials using non-disease modifying treatment, a disease modifying gene therapeutic treatment remains to be identified as effective in slowing or reversing PD disease progression.

In the investigation of growth factors for PD, researchers are challenged by the validity of the PD animal models used. For instance, 6-OHDA and MPTP-treated animals phenotypically display parkinsonistic symptoms, but the pathophysiology does not adequately mirror PD. This warrants more accurate PD models and their implementation in PD research. By utilizing the novel CRISPR-CAS9 technology it should be possible to engineer animals suffering mutations that result in true PD rather than an artificially induced one.

Another hurdle faced in exploring the use of growth factors. There must be some dopaminergic neurons left and if that can be saved by using the appropriate growth factors. At a longer perspective, gene therapy with growth factors could be relevant as an aggressive treatment regime in patients with early verified PD, rather than as a last-line of treatment.

Gene therapy with opto- and chemogenetics could prove a viable alternative option for treating symptoms of PD as they provide a more specific intervention as compared to DBS or ablation. Genome editing with the CRISPR-CAS9 technology might be another future form of personalized gene therapy for known mutations leading to PD.

Last but not the least; "gene-cell combo therapy" should be tried for clinical trial. The reason is that gene therapy may correct the faulty gene(s) and neural cell transplantation may replenish the loss of DA-ergic cells in the brain. Therefore, this combo treatment seems to be very logical for complete cure of the disease may appear at an early or late stage, or both.

In conclusion, while gene therapy has yet to deliver the true cure for PD, there is increasing data supporting that this treatment modality could become an important avenue for future PD treatment.

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