

# A Brief Overview of Tyrosine Hydroxylase and $\alpha$ -Synuclein in the Parkinsonian Brain

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**Abstract:** Parkinson's disease (PD) is associated with neurodegeneration of the nigrostriatal tract and is accompanied with loss of tyrosine hydroxylase (TH) and dopamine (DA). Development of neuroprotective strategies targeting PD is often undermined by lack of proper understanding of processes contributing to the pathology. In this mini review we have tried to briefly outline the involvement of TH and  $\alpha$ -synuclein in PD. Aberrant expression of  $\alpha$ -synuclein is toxic to dopaminergic neurons. It interacts with ubiquitin-proteasomal processing system, implicated in oxidative injury and mitochondrial dysfunction which ultimately induce neurodegeneration and cell death. The contributions of DJ-1 in TH regulation have also been discussed. Brain specific TH expression with the combined use of the pegylated immunoliposome (PILs) gene transfer technology and brain specific promoters as a new approach to treat PD has also been included.

**Keywords:** Tyrosine hydroxylase, dopamine, nigrostriatal pathway, alpha-synuclein, neurodegeneration, Parkinson's disease.

## INTRODUCTION

Each year, about 4 million to 6 million people suffer from Parkinson's disease (PD), with approximately 1 million in the USA alone. 4% of people with Parkinson's are diagnosed before the age of 50 and the incidence increases with age. Many experts think that the disease is caused by a combination of genetic and environmental factors (toxin) which may vary from person to person. Scientists have identified aging as an important risk factor which further increases the incidence by 1-2 % among people over the age of 50. In PD the neurodegeneration of the nigral striatal tract results in a loss of dopaminergic neurons in the substantia nigra (SN), a loss of tyrosine hydroxylase (TH) containing nerve endings in the striatum and diminished striatal dopamine (DA) production causing akinesia [1]. DA containing neurons seem to be the key players and their deficiency contributes to the typical clinical features of the disease which include tremor [2], bradykinesia [3], rigidity [4] and postural instability [4]. These symptoms can be temporarily treated by administering medications targeting DA metabolism and function, such as DA, precursor L-3,4-dihydroxyphenylalanine (L-DOPA) and DA agonists [5].

## TYROSINE HYDROXYLASE: LOCALISATION, STRUCTURE AND FUNCTION

Tyrosine hydroxylase and calmodulin are observed in almost all the brain regions with varying intensities. Highest levels of TH are observed in the caudate nucleus and putamen where its activity is probably being regulated by a

calcium/calmodulin-dependent system [6]. TH is the first rate limiting step in the biosynthesis of the catecholamines, DA, norepinephrine and epinephrine (Fig. 1). L-phenylalanine is converted into L-tyrosine by the action of the enzyme phenylalanine hydroxylase. TH then catalyzes the conversion of tyrosine to L-DOPA which is further metabolized into DA under the influence of aromatic amino acid decarboxylase (AADC) [7]. TH is also found in the adrenal gland [8]. Undoubtedly, a reduced 3, 4-dihydroxyphenylalanine production is observed in PD [9].

TH is an iron-containing stereospecific monooxygenase requiring tetrahydrobiopterin for its activity. Human TH exists in four isoforms (hTH1-4) that are produced by alternate splicing of mRNA from a single gene. The human TH gene consists of 14 exons [10]. The enzyme consists of a catalytic domain confined in the C terminal and making up almost 2/3rd of the molecule whereas the regulatory domain is present in the N-terminal region [11]. Its product L-DOPA can cross the protective blood-brain barrier (BBB) unlike DA. Thus, L-DOPA is given to increase DA concentrations in the treatment of PD and other abnormalities like DA-responsive dystonia [12]. Once L-DOPA has entered the central nervous system (CNS), it is converted into DA by the enzyme aromatic L-amino acid decarboxylase, also known as DOPA decarboxylase (DDC). Pyridoxal phosphate (vitamin B<sub>6</sub>) is a required cofactor in this reaction and may occasionally be administered along with L-DOPA, usually in the form of pyridoxine. However, vitamin B<sub>6</sub> is classically avoided in the treatment of PD, because it decreases the efficacy of L-DOPA. L-DOPA can be directly metabolized by catechol-O-methyl-transferase (COMT) to 3-O-methyldopa and then further to vanillic acid [7]. This metabolic pathway is homeostatically regulated in the healthy body, but becomes important after peripheral L-DOPA adminis-

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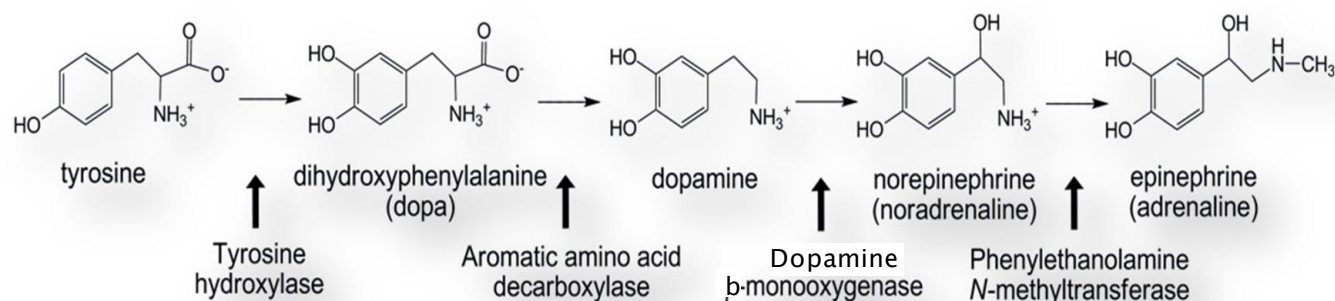


Fig. (1). Synthesis of catecholamines from tyrosine.

tration in patients with PD or in the rare cases of AADC deficiency.

### TYROSINE HYDROXYLASE: REGULATION AND DEFICIENCY

Briefly, TH activity is regulated in the short-term by feedback inhibition of catecholamines (epinephrine, norepinephrine and DA) which compete with tetrahydrobiopterin and by reversible phosphorylation at Ser-19 and Ser-40 in hTH1 [10]. Other isoforms of TH in humans have additional phosphorylation sites (Ser-8, Ser-31), providing means of further regulation. Short mechanisms thus control the activity of the preexisting forms of the enzyme. This phosphorylation can be catalyzed by a number of kinases, viz., protein kinase A (PKA), protein kinase C, calcium/calmodulin dependent protein kinase II and the MAP kinase, ERK. PKA mediated regulation of TH through Ser-40 modification has been extensively studied. Recently, it was observed that protein kinase C $\delta$  (PKC $\delta$ ) negatively regulates TH activity and DA synthesis. PKC $\delta$  is highly expressed in nigral dopaminergic neurons and co-localizes with TH. Interestingly, suppression of PKC $\delta$  activity with the kinase inhibitor rottlerin, PKC $\delta$ -small interfering RNA or with a PKC $\delta$  dominant-negative mutant effectively increased a number of key biochemical events in the DA pathway including TH-ser-40 phosphorylation, TH enzymatic activity, and DA synthesis in neuronal cell culture models. It is, therefore, suggested that PKC $\delta$ -mediated regulation of TH may have important implications in neurological dopaminergic system disorders such as PD [13]. The sequence Arg-37-Arg-38 of TH is known to play a significant role in feedback inhibition by the end product DA. This sequence has been shown to affect DA production more efficiently than the phosphorylated Ser-40 hTH1. This may provide yet another means of increasing the DA production *in vivo* [14] as well as other treatment strategies like administration of L-DOPA, DA agonists, inhibitors of DA metabolism, or a brain graft with cells expressing TH [15]. In long term regulations, like under stress conditions or repeated drug treatment, TH protein is induced. CRE (responsive to cAMP) in the 5' flanking region of the TH gene has been found to be one of the main functional elements for TH gene expression [10, 11]. Long term regulation in the adrenal medulla and locus coeruleus is mediated by modulation of TH gene transcription and mRNA stability [16].

TH deficiency is a rare autosomal recessive disorder mapped to chromosome 11p15.5. It is clinically manifested as dopa-responsive dystonia and juvenile Parkinsonism [17]. Reports are available regarding TH deficiency and an early onset of progressive L-dopa-responsive dystonia [18-20]. Neonates are recognized with a more severe phenotype described as progressive, L-dopa-non-responsive encephalopathy [21]. TH deficiency leads to low concentrations of L-DOPA and a consequent decline in levels of catecholamines. TH deficiency can be diagnosed by demonstrating decreased CSF levels of the downstream metabolites of the catecholamine degradation pathway i.e. homovanillic acid and 3-methyl-4-hydroxyphenyl ethylene glycol (MHPG) and by mutation analysis of the TH gene [7].

TH deficiency is almost exclusively caused by missense mutations in the TH gene and its promoter region, suggesting that mutations with more a deleterious effect on the protein are incompatible with life. The clinical features of TH deficiency such as tremor, hyperkinesia, bradykinesia, rigidity and dystonia result from cerebral DA and norepinephrine deficiency [7]. As the clinical symptoms of TH deficiency often overlap with other neurological disorders, the diagnosis of the disease is not easy and requires extensive testing including cerebral imaging, CSF composition and L-DOPA responsiveness analysis and screening of inborn errors of metabolism [7]. Besides treatment with L-DOPA, alternate treatments strategies are underway trials. Modification of the DNA encoding the enzyme TH resulting in amino acid substitution in one of the first fifty five N-terminal residues, in particular replacing Ser-40 with Tyr or Leu, produced TH variants having substantially increased enzymatic activity upon transfection of suitable host cells. Cells transfected with the variant TH having enhanced enzymatic activity can be grafted into the brain and may prove useful for treating neurological or psychiatric disorders associated with deficient TH or DA, in particular, PD [22].

In PD there is a selective loss of dopaminergic neurons with decreased striatal DA level [23]. DA is a catecholamine neurotransmitter that participates in modulating diverse brain processes, including those that influence motor function, emotions and addiction [24]. Reduction in DA level has been associated with many disorders of the CNS, including Huntington's chorea and PD. PD is associated with a massive loss in melanized dopaminergic neurons in the substantia nigra resulting in a severe striatal dopaminergic

denervation. The hyperactivity which develops in the remaining striatal dopaminergic terminals may be related to an increased rate of tyrosine hydroxylation. This could be related to changes in the level of expression of the gene coding for TH. Thus, the detection of TH messenger RNA was looked for at cellular levels by *in situ* hybridization histochemistry. Image analysis showed that the hybridization signal was significantly reduced in the surviving neurons when compared to control. The subnormal TH messenger RNA content may express a change in level of TH gene transcription, possibly in relation to sustained suffering of the neurons still present at late stages of the disease [25].

### ALPHA SYNUCLEIN AFFECTING TH IN PD

Information regarding pathogenesis of PD is lacking. Various factors that may contribute to the disease onset and progression have been summarized in Fig. (2). One such contributing factor is  $\alpha$ -synuclein ( $\alpha$ -syn), a protein highly enriched in presynaptic terminals [26] and implicated in normal brain function [27, 28]. Although aberrant expression of  $\alpha$ -syn is toxic to dopaminergic neurons, little is known about the correlation between abnormality of  $\alpha$ -syn and the expression of TH [29]. Human  $\alpha$ -syn, a 140 amino acid protein highly expressed in the CNS [30, 31], was originally identified in Alzheimer's disease brains and has been strongly implicated in the pathogenesis of PD [32]. Katrina Gwin indicated that 13 gene mutations are associated with PD. Basically there are two categories of genes which are associated with this disease. The first "causal genes" actually

cause the disease. The second "associated genes" do not cause PD on their own, but increase the risk of developing it.  $\alpha$ -syn is a causal gene located on chromosome 4 [33]. Over expression of  $\alpha$ -syn has been found to inhibit TH activity and DA synthesis in transgenic mice and  $\alpha$ -syn transfected dopaminergic neurons [34, 35]. Overexpression of  $\alpha$ -syn reduces the protein level of TH and inhibits TH gene expression without affecting the cell growth and proliferation, and induces cell injury in  $\alpha$ -syn transfected MES 23.5 dopaminergic neuronal cell line [29]. These findings are indicative of the fact that inhibition of TH gene expression may be directly due to aberrantly expressed  $\alpha$ -syn in dopaminergic cells.

The exact mechanism for the inhibition of the TH gene expression in  $\alpha$ -syn gene-transfected dopaminergic cells is not clear. Gene transfection itself may not be the cause of the loss of TH expression as its expression was not affected in vector control cells. Therefore, overexpressed human  $\alpha$ -syn in dopaminergic cells may be responsible for the loss of TH expression. Similar results have been observed in human  $\alpha$ -syn transgenic mice, in which both the TH level and TH activity in striatum were decreased [34]. In PD, the proteasomal system  $\alpha$ -syn degradation has been demonstrated to be deficient [36, 37], which can also result in the intracellular accumulation of  $\alpha$ -syn. Polymorphism in the promoter region of the  $\alpha$ -syn gene can affect the level of expression of  $\alpha$ -syn, and some of the alleles are related to a high risk of PD onset [38]. Therefore it is postulated that both genetic deficiency and environmental risk factors may

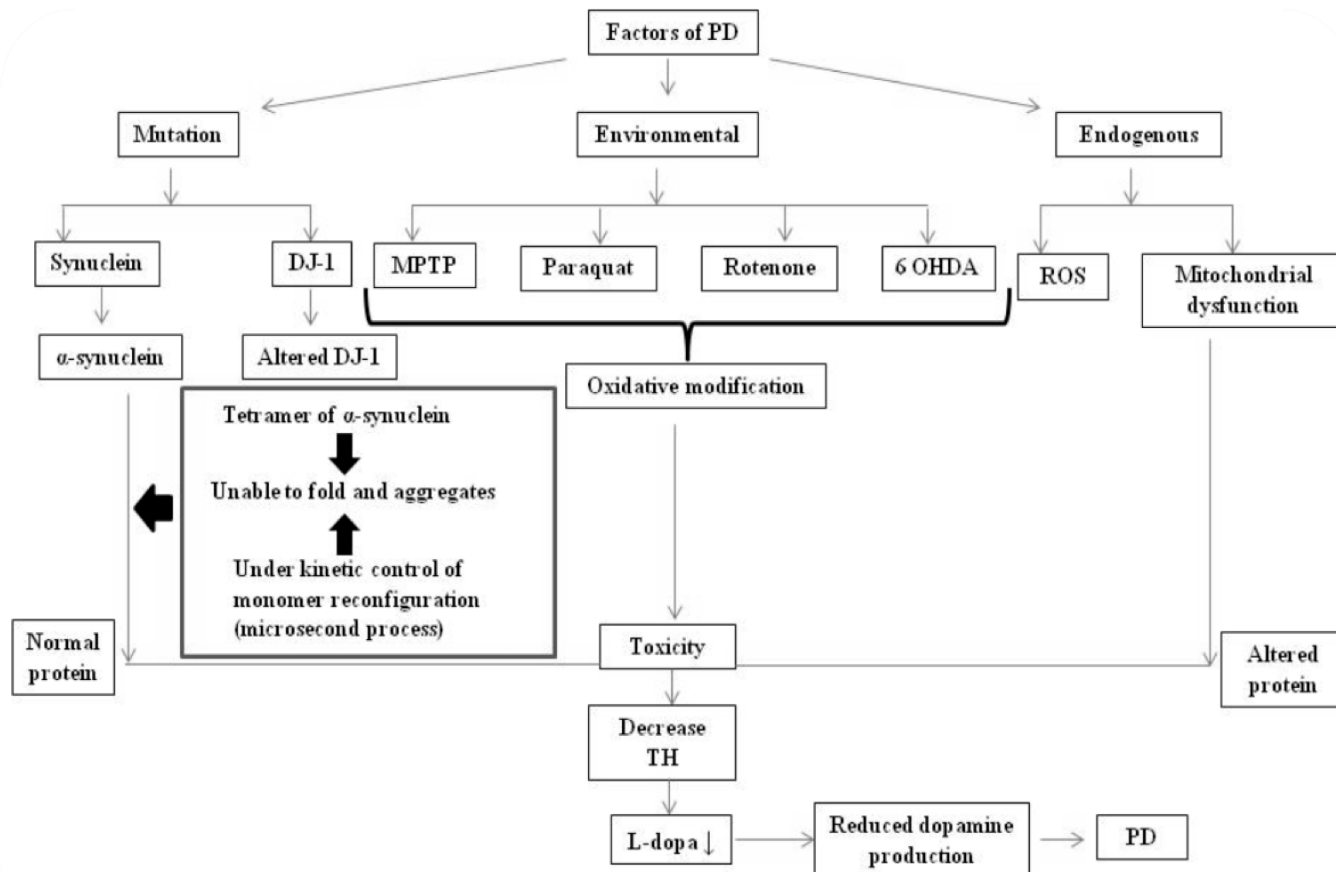


Fig. (2). Scheme depicting the routes to pathogenesis of PD.

work together to induce accumulation of  $\alpha$ -syn in dopaminergic neurons, which leads to the inhibition of both expression and activity of TH [29]. Findings show that  $\alpha$ -syn regulates DA synthesis by binding to and inhibiting TH in DA synthesis. As already discussed short-term regulation of TH depends on the phosphorylation of key seryl residues in the amino-terminal regulatory domain of the protein. Of these, Ser-40 contributes significantly to TH activation and DA synthesis.  $\alpha$ -syn overexpression caused a reduction in Ser-40 phosphorylation in MN9D cells and inducible PC12 cells. Ser-40 is phosphorylated chiefly by the cyclic AMP-dependent protein kinase PKA and is dephosphorylated almost exclusively by the protein phosphatase, PP2A. Therefore, impact of  $\alpha$ -syn overexpression on levels and activity of PKA and PP2A was measured in the cells. It was found that inhibition of PP2A dramatically increased Ser-40 phosphorylation only in  $\alpha$ -syn overexpressors. These findings reveal a functional interaction between  $\alpha$ -syn and PP2A that leads to PP2A activation and underscores a key role for  $\alpha$ -syn in protein phosphorylation [39].

*In vitro* studies have shown that  $\alpha$ -syn may interact with ubiquitin-proteasomal processing [40], oxidative stress pathways injury and/or cause mitochondrial dysfunction to induce neurodegeneration and cell death [41, 42]. Moreover it has been recently reported that transgenic mice expressing human wild type or mutant  $\alpha$ -syn present human features of PD, including loss of DA in striatum, motor impairment, formation of  $\alpha$ -syn positive inclusions and neuronal degeneration [43, 34].

Direct evidence for involvement of  $\alpha$ -syn in PD was provided by genetic studies in which mutations in  $\alpha$ -syn gene (A53T and A30P) cause rare, dominantly inherited variations of this disorder [44, 45]. Although the effort to find these mutations in the  $\alpha$ -syn gene in sporadic PD cases failed [46, 47],  $\alpha$ -syn is found to be the primary component of intracellular aggregates known as Lewy bodies and Lewy neurites, a pathological hallmark of PD and likely plays a central role in neuronal death [48-51].  $\alpha$ -syn is highly prone to aggregation [52] and the purified protein has been shown to exist in an intrinsically disordered state in solution. A30P  $\alpha$ -synuclein, a mutant, has been found to be associated with early onset PD. It has significantly altered conformational properties, shows rapid nucleation (bimolecular association) but slow fibrillation, unlike the wild type protein. This supports the idea that the disease is caused by the earlier stages of aggregation and these stages are controlled by intramolecular diffusion.

#### NEUROTOXIC AGENTS AND DJ-1: RELATION WITH TH AND PD

A synthetic neurotoxic agent MPTP can also cause immediate and permanent Parkinsonism. The incidence of MPTP-induced Parkinson's in the general population is exceedingly rare. It is believed that environmental toxin is never enough to cause Parkinson's. However, MPTP and the herbicide paraquat can up-regulate  $\alpha$ -syn expression [53, 54]. Experiments have shown that MPTP, 1-methyl-4-phenylpyridinium ion (MPP<sup>+</sup>) and structurally related compounds including N-methyl tetrahydroisoquinilinium ion, inhibit the

TH system in tissue slices of rat striatum. The results with the structurally related compounds of MPP<sup>+</sup> indicate that both the pyridinium and the phenyl group are required for this inhibition [55]. Chronic rotenone exposure *in vivo* causes oxidative modification of DJ-1, accumulation of  $\alpha$ -syn, and proteasomal impairment which leads to degeneration of the nigrostriatal pathway [56].

Loss of functional mutations in DJ-1 causes a subset of familial PD. However the mechanism underlying the selective vulnerability in dopaminergic pathway due to inactivation of DJ-1 is unclear. DJ-1 is a neuroprotective transcriptional co-repressor pyrimidine tract binding protein associated with splicing factor (PSF). DJ-1 and PSF bind and regulate the human TH. Promoter inactivation of DJ-1 by small interfering RNA (siRNA) results in decreased TH expression and L-dopa production in human dopaminergic cell lines. Consistent with its role as a transcriptional regulator, DJ-1 specifically suppresses the global small ubiquitin like modifier (SUMO-1) modification. High molecular weight sumoylated protein species including PSF, accumulate in the lymphoblast cells of the patients carrying pathogenic DJ-1 mutations [5]. DJ-1 elevates TH expression by inhibiting the sumoylation of PSF and preventing its sumoylation-dependent recruitment of histone deacetylase1. Furthermore, siRNA silencing of DJ-1 decreases the acetylation of TH promoter-bound histones, and histone deacetylase inhibitors restore the DJ-1 siRNA-induced repression of TH. This suggests that DJ-1 is a regulator of protein sumoylation and directly linked the loss of DJ-1 expression and transcriptional dysfunction to impaired DA synthesis. The formation of cysteine-sulfinic acid has recently been appreciated as a modification that links protein function to cellular oxidative status. Human DJ-1 readily forms cysteine-sulfinic acid at a conserved cysteine residue (Cys-106 in human DJ-1). Mutation of Cys-106 causes the protein to lose its normal protective function in cell culture and model organisms. To prove this a series of substitutions were made at a proximal glutamic acid residue (Glu18) in human DJ-1 that alter the oxidative propensity of Cys-106 through changes in hydrogen bonding. It was found that two mutations, E18N and E18Q, allow Cys-106 to be oxidized to Cys<sup>106</sup>-sulfinic acid under mild conditions. Therefore E18N and E18Q can both partially substitute for wild-type DJ-1 using mitochondrial fission and cell viability assays. Finally it was concluded that formation of Cys<sup>106</sup>-sulfinic acid is a key modification that regulates the protective function of DJ-1 [57].

Loss of functional mutations in the DJ-1 gene cause early onset of Parkinsonism [58]. The evolutionarily conserved DJ-1 has been shown to regulate oxidative stress, apoptosis, protein aggregation, and transcription in various subcellular compartments. The DJ-1 gene encodes a ubiquitous, highly conserved protein. DJ-1 mutations are associated with Park 7, a monogenic form of human Parkinsonism. The function of DJ-1 protein remains unknown, but evidence suggests it is involvement in the oxidative stress response [59]. Further findings indicate that loss of DJ-1 function leads to neurodegeneration. Elucidating the physiological role of DJ-1 protein may promote understanding of the mechanisms of brain neuronal maintenance and pathogenesis of PD.

## TARGETING PD

For Parkinson's treatment some prefer levodopa preparation while others go for the agonists, an MAO inhibitor or an anticholinergic. Medications available to treat Parkinson's in the market include carbidopa/levodopa formulation which is called Sinemet. This formulation is very effective as carbidopa prevents levodopa from being converted into DA in the blood stream, allowing more of it to go to brain. Therefore, a smaller dose of levodopa is needed to treat symptoms. Unfortunately, with increased dosing and prolonged use of this drug, patients experience other side effects including dyskinesias. Autografting of DA-producing adrenal medullary tissue to the striatal region of the brain is being attempted in patients with PD. It was observed that in the putamen there was a nearly complete depletion of DA in all subdivisions, with the greatest reduction accounted for less than 1% in the caudal portions. Therefore caudal portions of putamen may be the most appropriate for intrastriatal application of DA-producing autografts in patients with idiopathic PD [60].

Transvascular gene therapy of PD is a new approach to gene therapy of PD and involves the global distribution of a therapeutic gene to brain cells after an intravenous administration and transport across the blood-brain barrier (BBB) [61]. This is enabled with the development of a non viral gene transfer technology that encapsulates plasmid DNA inside pegylated immunoliposomes or PILs. An 85- to 100-nm liposome carries the DNA inside the nanocontainer, and the liposome surface is conjugated with several thousand strands of 2000-Da polyethyleneglycol (PEG). This PEGylation of the liposome stabilizes the structure in the blood stream. The liposome is targeted across the BBB *via* attachment to the tips of 1-2% of the PEG strands of a receptor-specific monoclonal antibody (mAb) directed at a BBB receptor, such as the insulin receptor or transferrin receptor (TfR). Owing to the expression of the insulin receptor or the TfR on both the BBB and the neuronal plasma membrane, the PIL is able to reach the neuronal nuclear compartment from the circulation. Brain-specific expression is possible with the combined use of the PIL gene transfer technology and brain-specific gene promoters. In the 6-hydroxydopamine rat model of experimental PD, striatal TH activity is completely normalized after an intravenous administration of TfRmAb-targeted PILs carrying a TH expression plasmid. A treatment for PD may be possible with dual gene therapy that seeks to replace striatal TH gene expression with the TH gene.

Parkinson's disease results from a selective loss of DA-releasing neurons. Since L-DOPA as a compensatory source of DA has a number of undesired side effects as well, Dr. Goldstein and his co-workers have used molecular genetic techniques to enhance the activity of TH by inducing genetic modification at key amino acid residues. Genetic constructs encoding variant TH enzymes can be transfected into cells which will subsequently express TH with enhanced activity. These transfected cells will provide a source of cells for transplantation to treat conditions associated with defective function of the TH enzyme. Genetic constructs of TH and transfected cells expressing enhanced TH activity provide a novel therapeutic approach for treating Parkinson's and Alzheimer's diseases [62].

## CONCLUSIONS

Neurodegeneration of the nigrostriatal tract accompanied with loss of DA and TH results in PD with clinical symptoms including tremor, bradykinesia, rigidity and postural instability. Aberrant expression of  $\alpha$ -syn is toxic to dopaminergic neurons and has been strongly implicated in the pathogenesis of this disease. Promoter inactivation of DJ-1 by siRNA results in decreased TH expression and L-dopa production in human dopaminergic cell lines. Formation of Cys<sup>106</sup>-sulfenic acid is a key modification that regulates the protective function of DJ-1 and loss of its function leads to neurodegeneration and onset of Parkinsonism. MPTP, rotenone and the herbicide paraquat can also upregulate  $\alpha$ -syn expression and hamper TH synthesis. Therefore it is surmised that both genetic deficiency and environmental risk factors may work together to induce accumulation of  $\alpha$ -syn in dopaminergic neurons, which leads to the inhibition of both expression and activity of TH [29]. The caudal portion of the putamen is found to be the most appropriate part of the brain for interstitial application of DA-producing autografts in patients with idiopathic PD. Brain specific expression is possible with the combined use of PIL gene transfer technology and brain specific promoters. Therefore, treatment of PD may be possible with dual gene therapy that seeks to replace striatal TH gene expression with the TH gene. Genetic constructs of TH with modified key amino acid and transfected cells expressing enhanced TH can be used for the treatment of PD.

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Declared none.

## CONFLICT OF INTEREST

Declared none.

## ABBREVIATIONS

|               |   |  |
|---------------|---|--|
| $\alpha$ -syn | = | Alpha synuclein                                |
| AADC          | = | Aromatic L-aminoacid decarboxylase             |
| BBB           | = | Blood brain barrier                            |
| COMT          | = | Catechol-o-methyl transferase                  |
| DOPA          | = | 3,4-Dihydroxy phenylalanine                    |
| DA            | = | Dopamine                                       |
| DDC           | = | Dopa decarboxylase                             |
| MHPG          | = | 3 Methyl -4- hydroxyl phenylethylene glycol    |
| MPP+          | = | 1, Methyl-4-phenyl-pyridinium ion              |
| MPTP          | = | 1-methyl-4-phenyl-1, 2, 3,6-tetrahydropyridine |
| PD            | = | Parkinson's disease                            |
| PIL           | = | pegylated immunoliposome                       |
| ROS           | = | Reactive oxygen species                        |
| siRNA         | = | Small interfering RNA                          |
| SNpc          | = | Substantia nigra pars compacta                 |
| TfR           | = | Transferrin receptor                           |
| TH            | = | Tyrosine hydroxylase                           |

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