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# Role of SNCA Gene Mutations and Alpha-Synuclein Pathology in Parkinson's Disease: Aav2-Gad Gene Therapy

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**ABSTRACT**

A progressive neurodegenerative ailment, Parkinson's Disease causes motor deficits and impairment in movement due to Alpha-Synuclein protein aggregates in the brain, which gradually worsen over time. The protein Alpha-Synuclein is encoded by the SNCA gene which is mutated in cases of familial Parkinson's and leads to abnormal aggregation of Alpha-Synuclein. This paper studies the role of mutations in the SNCA gene and Alpha-Synuclein pathology in the progression of PD and assesses the therapeutic potential of administration of the glutamic acid decarboxylase (GAD) gene through adeno-associated virus type 2 (AAV2).

## INTRODUCTION

Parkinson's Disease (PD) is a neurodegenerative disease whose first clinical symptoms were detected by James Parkinson in 1817, and was previously known as "shaking palsy". Abnormal protein aggregates known as Lewy Bodies comprising the protein Alpha-Synuclein (α-Synuclein) get accreted in neurons. α-Synuclein is a protein found inside the neurons specifically located in the substantia nigra in the brain. Its main function appears to be regulating dopamine and its release through effects in the SNARE (Soluble N-ethylmaleimide-sensitive factor activating protein receptor), however, its precise function still remains mysterious. The α-Synuclein proteins in the neuron come together and form chains which are transient. Neurons are equipped with molecular chaperones and a ubiquitin-proteasome system which are essential for autophagy, the effective functioning of the neurons to balance and manage the chained α-Synucleins. In some cases, these chains may become too abundant, or, the efficacy of the ubiquitin-proteasomes and molecular chaperones may decrease. The α-Synuclein chains in these cases merge together into protofibrils which form aggregates that, eventually, become Lewy Bodies. The presence of Lewy Bodies in neurons affects the ideal functioning of the neuron and therefore causes them to degenerate. Lewy Bodies proceed to spread from the substantia nigra to the hypothalamus, cerebral cortex and locus coeruleus.

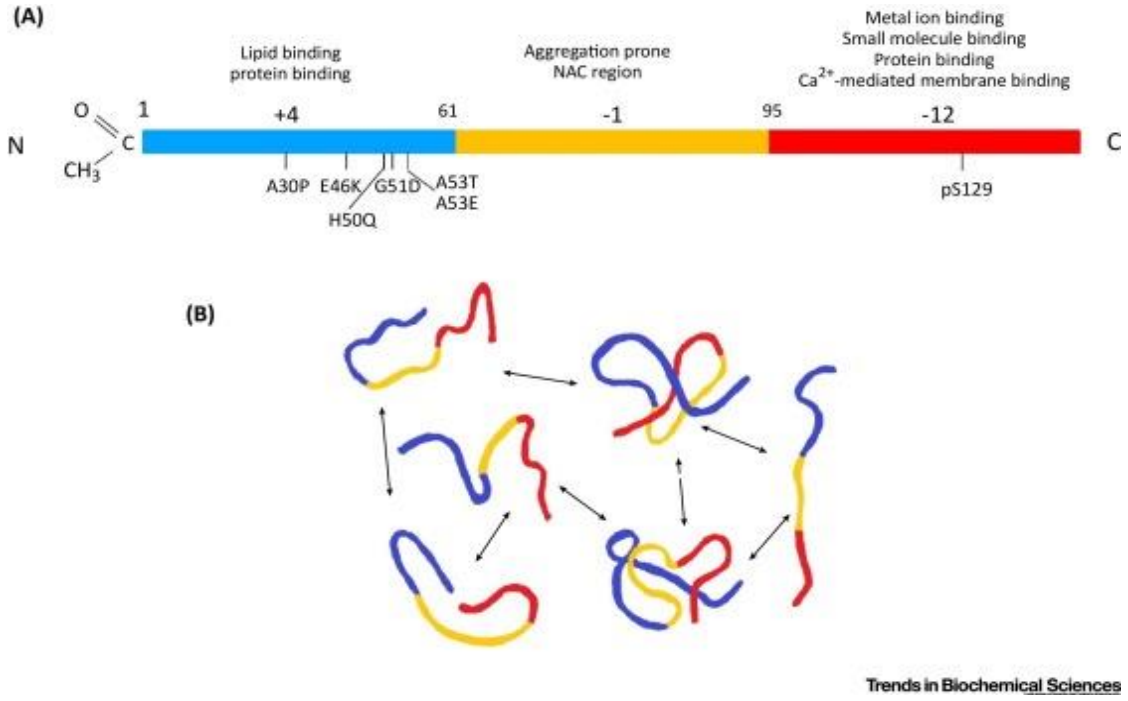


**Fig 1. Structure of α-Synuclein protein**

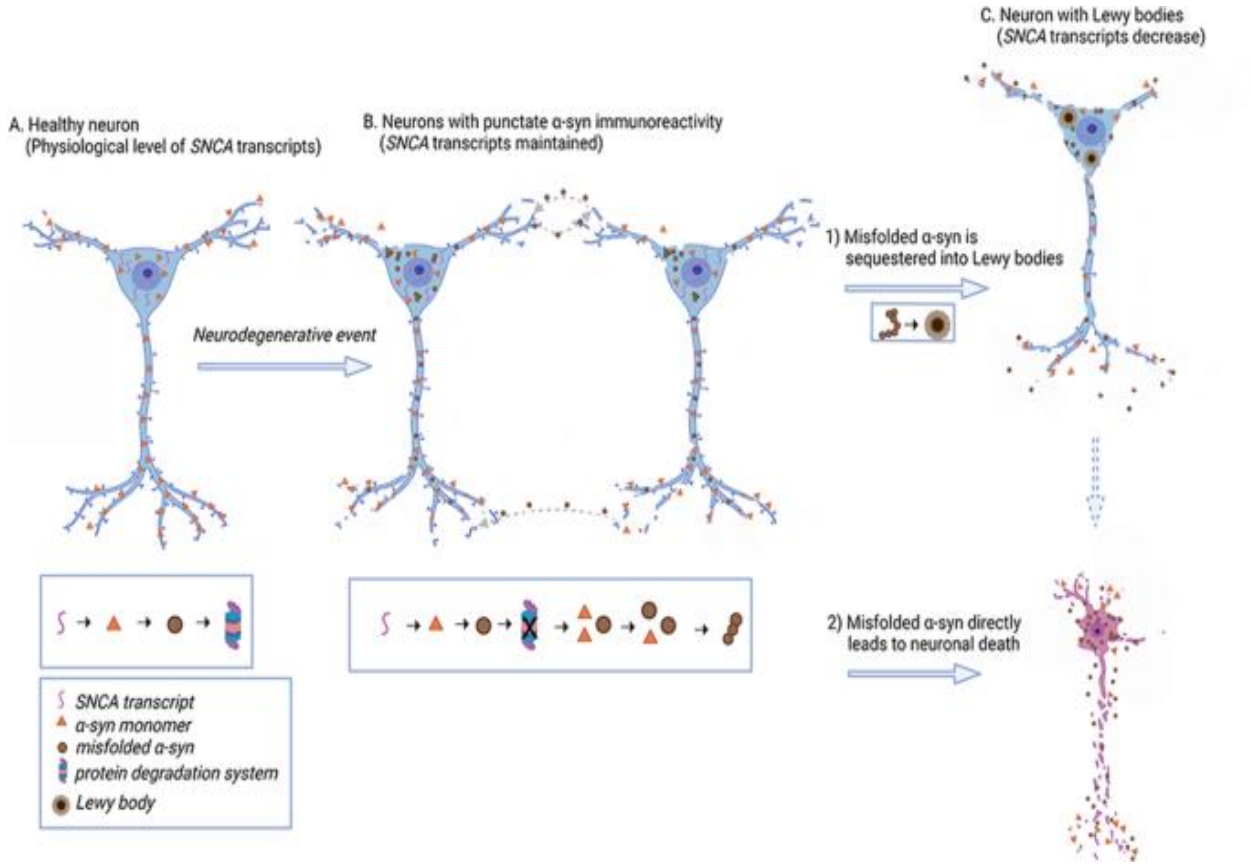


**Fig 2. Progression of degeneration of neuron in PD**

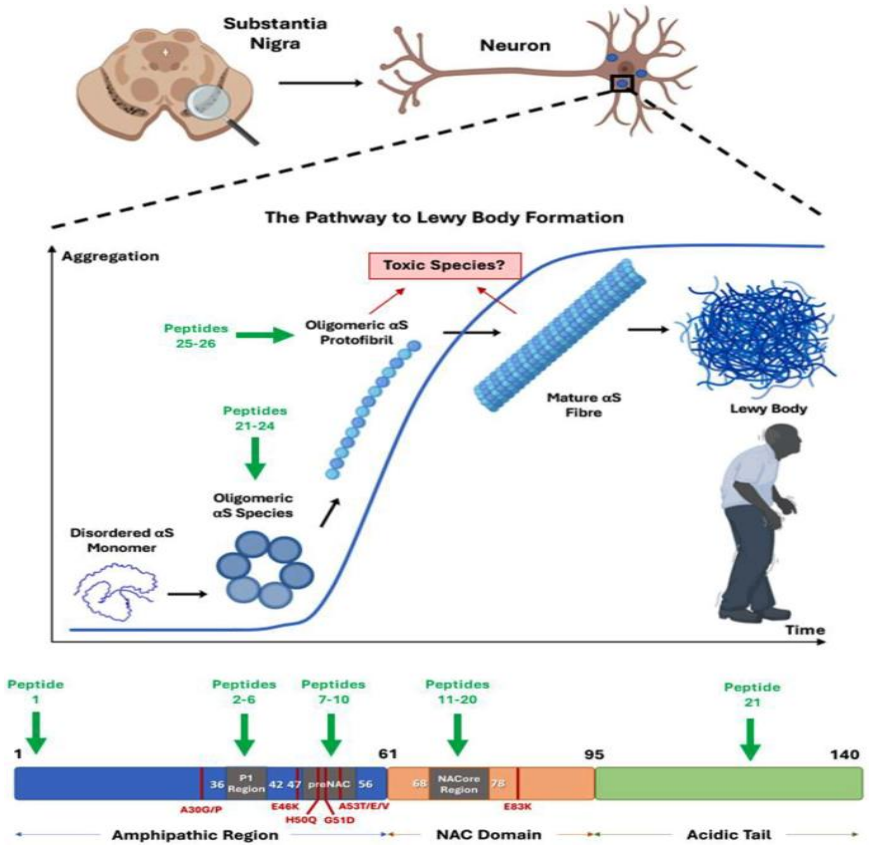


**Fig 3. αS aggregation into Lewy bodies in PD and regions of interest. Bottom: the primary structure of αS is shown divided into three distinct regions.**

The neurons in the substantia nigra are characterised by movement control and they directly communicate with the nerve cells in the basal ganglia through symmetrical synapses. Neurons in the substantia nigra liberate the neurotransmitter dopamine DA (4-(2-aminoethyl)benzene-1,2-diol) to the basal ganglia and this interaction affects the movement of the organism. DA is responsible for regulation of movement and also inducing a feeling of pleasure or satisfaction in the body. It is produced in the substantia nigra as well as the ventral tegmental area and hypothalamus located in the brain. As the neurons in the substantia nigra degenerate, the amount of DA viable for transmission in the corpus striatum in the brain is significantly lowered. This chemical imbalance starts manifesting through clinical symptoms which can be motor or non-motor. Motor dysfunctions of PD include bradykinesia, gait trouble, stuttering in speech, dystonia, muscle rigidity, impaired balance. Non-motor dysfunctions of PD include bradycardia, anxiety, disturbance in sleep, pain/numbness/tingling sensation in affected limbs, constipation, bradyphrenia and a decline in cognitive abilities.

Genetic links have been found in PD and it has been proven that 10-15% of PD patients inherit this disease. These cases are referred to as familial PD. In cases of familial PD, it is inherited in an autosomal dominant or autosomal recessive pattern.

23 chromosomal regions in the DNA helix are related to PD out of which 6 contain mutations that cause monogenic PD, where a mutation in one single gene can cause a change in the phenotype. 23 specific chromosomal regions, also known as chromosomal loci, contain PD mutations and these regions are known as PARK. Numbered in a chronological order, they are identified as PARK1, PARK2, PARK3,

PARK4, PARK5, and so on till PARK23. It was later discovered that chromosomal locus PARK4, regarded as the locus associated with PD, was identical to locus PARK1 (SNCA gene).

| PARK | Gene | OMIM reference | Inheritance | Description | Clinical features |
|---|---|---|---|---|---|
| PARK1 PARK4 | *SNCA* (44,45,57) | 168601 | AD | α-synuclein | Ranging from classical PD to early-onset cases with dementia, autonomic dysfunction, and rapid progression |
| PARK2 | *PRKN* (58) | 600116 | AR | parkin RBR E3 ubiquitin protein ligase | Early-onset PD, slow progression, often features of dystonia |
| PARK5 | *UCHL1* (59) | 613643 | AD | ubiquitin C-terminal hydrolase L1 | Classical PD—only one family, findings not since replicated |
| PARK6 | *PINK1* (60) | 605909 | AR | PTEN-induced putative kinase 1 | Early-onset PD, slow progression |
| PARK7 | *DJ-1* (61) | 606324 | AR | Parkinsonism-associated deglycase | Early-onset PD, slow progression |
| PARK8 | *LRRK2* (62) | 607060 | AD | Leucine-rich repeat kinase 2 | Classical PD with less frequent dementia and slower progression |
| PARK9 | *ATP13A2* (53) | 606693 | AR | Cation-transporting ATPase 13A2 | Early-onset (adolescence), atypical parkinsonism with dementia, spasticity and supranuclear palsy (Kufor–Rakeb syndrome) (63) |
| PARK11 | *GIGYF2* (64) | 607688 | AD | GRB10 interacting GYF protein 2 | Classical PD |
| PARK13 | *HTRA2* (65) | 610297 | AR | HtrA serine peptidase 2 | Classical PD |
| PARK14 | *PLA2G6* (54) | 612593 | AR | Calcium-independent phospholipase A2 enzyme | Early onset with atypical features (dystonia parkinsonism) |

| | | | | | |
|---|---|---|---|---|---|
| PARK15 | *FBX07* (55) | 260300 | AR | F-box protein 7 | Early onset with atypical features (pallido-pyramidal syndrome) |
| PARK17 | *VPS35* (66) | 614203 | AD | Vacuolar protein sorting-associated protein 35 | Classical PD |
| PARK18 | *EIF4G1* (67) | 614251 | AD | Eukaryotic translation initiation factor 4 gamma 1 | Classical PD |
| PARK19 | *DNAJC6* (56) | 615528 | AR | HSP40 Auxilin | Early-onset PD, slow progression |
| PARK20 | *SYNJ1* (68) | 615530 | AR | Synaptojanin 1 | Parkinsonism with dystonia and cognitive decline |
| PARK21 | *DNAJC13* (69) | 616361 | AD | Receptor-mediated endocytosis 8 (RME-8) | Classical PD |
| PARK23 | *VPS13C* (70) | 616840 | AR | Vacuolar protein sorting-associated protein 13C | Early-onset PD, rapid progression |

(OMIM: Online Mendelian Inheritance in Man database, AD: autosomal dominant, AR: autosomal recessive.)

**Table 1. PARK-designated genes involved in familial Parkinson's disease**

10 common genes associated with PD include GBA (Glucocerebrosidase), SNCA (synuclein-alpha), LRRK2 (Leucine-Rich Repeat Kinase 2), PRKN, PINK1 (PTEN-Induced Putative Kinase 1), DJ-1 (Protein Deglycase DJ-1), VPS35 (Vacuolar Protein Sorting 35), UCHL1 (Ubiquitin Carboxyl-Terminal Hydrolase L1), ATP13A2 (ATPase Cation Transporting 13A2) and EIF4G1 (Eukaryotic Translation Initiation Factor 4 Gamma 1**)** out of which GBA is the most common PD related gene. The GBA gene located on chromosome 1q21 is involved in encoding the glucocerebrosidase enzyme which is responsible for lysosomal hydrolysis. Mutations in this gene are found in 5-10% of PD patients and can cause accretion of α-Synuclein in the brain. Ashkenazi Jews are the most common PD patients affected by GBA mutations. There are 6 monogenic genes. SNCA, LRRK2, Parkin (PRKN), PINK1, DJ-1, and ATP13A2 are the 6 monogenic genes in which PD mutations occur. Mutations in SNCA (PARK1=4) and LRRK2 (PARK8) cause autosomal dominant PD and mutations occurring in the PRKN (PARK2), DJ-1 (PARK7), ATP13A2 (PARK9) and PINK1 (PARK6) genes cause autosomal recessive PD. Autosomal Dominant PD occurs when only one copy of the mutated gene inherited from the parent is enough to develop ADPD in the offspring. In Autosomal Recessive PD, both alleles of the chromosome must be mutated for the offspring

to attain the disorder.

**SNCA**

The SNCA (Synuclein Alpha) gene is located on PARK1 and PARK4 on the long arm of chromosome 4 (4q21.3-22). It is responsible for encoding α-Synuclein, the key protein responsible for the development of PD. SNCA was the first gene in which mutations were recorded that caused autosomal dominant PD, which also responded well to Levodopa (l-DOPA) treatment. Since PD is characterised by the reduction in amount of DA, l-DOPA (l-3,4-dihydroxyphenylalanine) is a drug prescribed to patients which acts as a precursor to DA. It is absorbed by neurons and chemically converted to DA in the brain.

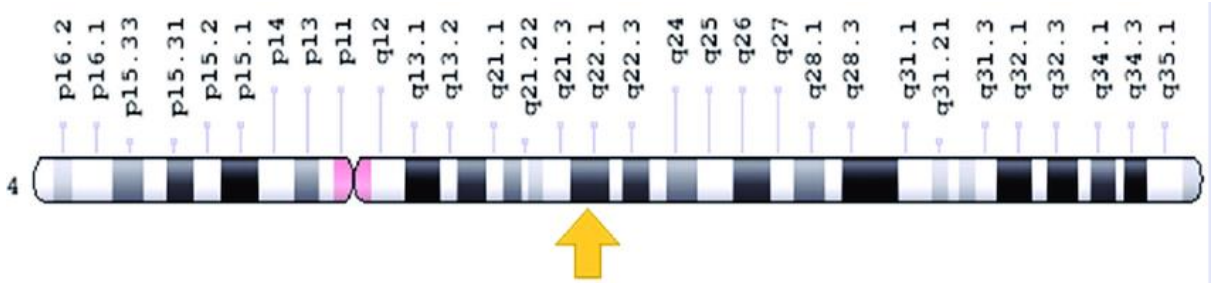


**Fig 4. Locus of SNCA gene**

Patients bearing SNCA mutations are often diagnosed with early onset PD (below the age of 50). The first mutation linked to PD was observed in a large Italian family and a few Greek kindred by Polymeropoulos in 1997. The mutation entailed G209A substitution (Guanine to Adenine substitution at nucleotide 209) in SNCA which eventually led to A53T (Alanine to Threonine substitution at nucleotide 53) amino acid change. A53T is a missense mutation, which means that a nucleotide base in a DNA sequence is swapped for another one, causing the development of a different amino acid. A53T mutation can be understood by saying that the 53rd amino acid is changed from alanine to threonine as a result of the conversion of guanine to adenine at position 209 in SNCA. Two other mutations, A30P (substitution of alanine to proline at nucleotide 30) and E46K (glutamic acid to lysine substitution at nucleotide 46) were also identified, however, the A53T mutation is the most commonly found. It was found in eight Greek families, two Korean, one Italian and one Swedish family.

It has been proved that A30P mutations in SNCA caused an early onset of PD and were also prescribed a milder course as compared to the patients who were affected by the A53T mutation.

The A30P point mutation was first discovered in a German family which displayed substitution of alanine to proline at position 30 of the nucleotide. In this point mutation, α-Synuclein is incapable of connecting the N-terminal, the beginning of a polypeptide that consists of singular sequences of peptides which directs the protein to the required organelle, to the synaptic vesicles which release neurotransmitters. This mutation turns α-Synuclein pathogenic and prone to disease. It also halts autophagic flux by inactivating JNK (c-Jun N-terminal kinase) signalling, a protein pathway that alters and regulates cellular activities and autophagy, located in the midbrain dopaminergic (mDA) neurons in the ventral midbrain. Additionally, this mutation also decreases protein LC3 (Microtubule associated proteins 1A/1B light chain 3) that regulates the number of autophagosomes and increases levels and activity of the protein ZKSCAN3 (Zinc Knuckle and SCAN Domain Containing 3) which is an autophagy repressor. These are the results of the A30P mutation which therefore lead to the building up, oligomerization and fibrillation of α-Synu-

clein into amyloid fibrils.

The E46K mutation which substitutes Glutamic Acid to Lysine at nucleotide 46 was first detected in an autosomal dominant PD suffering Spanish kindred. In a recent study, researchers used cryo electron microscopy (cryo-EM) and NMR (Nuclear Magnetic Resonance) to determine that the E46K mutation causes morphological changes in the fibril structures of α-Synuclein in the neurons. The study showed that it alters the stability and conformation of the α-Synuclein fibrils, revealing that the E46K mutated α-Synuclein has a twice as more twisted structure than an unmutated α-Synuclein fibril with a right sided twisted helical morphology as opposed to the left handed twist in unmutated α-Synuclein morphology. Under freezing and sonication conditions, the E46K mutated α-Synuclein broke apart into smaller and shorter fragments as compared to the unmutated α-Synuclein fibril which revealed that the mutated α-Synuclein is much more unstable than the unmutated fibril therefore, more prone to pathogens. E46K mutations also rearrange electrostatic and polarisation interactions in α-Synuclein.

The A53T mutation results in early onset of PD. It was first observed in a Greek and Italian family, as well as a Korean family. This mutation occurs in the N-terminal of the α-Synuclein protein and causes the N or C terminals to disappear, making it slightly similar to the A30P mutation. They cause formation of annular and tubular protofibrils and get more tightly bound to phospholipid vesicles which eventually result in permeabilization. It promotes fibrillization of the protein. This mutation also makes the organism more vulnerable to DA toxicity, which can inhibit Mitochondrial Complex 1. Mitochondrial Complex 1 carries out oxidative phosphorylation and provides the cells with energy and DA toxicity inhibits this process. DA toxicity can also cause further ailments like schizophrenia, ADHD and psychosis.

A53T mutations also suppress and deplete the presynaptic transmission in neurons. It causes the depletion of the synaptic vesicle recycling pool. The recycling pool retrieves fused vesicle membranes to generate new vesicles, which is destroyed by the A53T mutation.

Inflammation caused by the A53T mutation brings about mitochondrial and endoplasmic reticulum (ER) stress which is characterised by mitochondrial dysfunction- the loss of efficiency of the electron transport chain (ETC) and decrease in production of energy rich molecules like ATP. This could possibly result in mitochondrial autophagy which can further lead to neurodegeneration.

**GENE THERAPY: AAV2-GAD**

DNA being the primary biomolecule for transfer of genetic information and genetic inheritance allowed researchers to delve further into researching how mutated genes can be modified and amended to favour humans. If mutated genes could be altered at a molecular level or expression of genes could be regulated, chances of a disease being potentially cured are high.

There have been recent discoveries regarding the use of viruses for gene therapy. The natural and biological function of a virus is to deliver its own DNA/RNA into the host cell in order to get replicated by the host cells. Researchers have evaluated this function of viruses to use it for gene therapy. This technique involves viral vectors, which are modified viruses, to deliver genetic material into the host cells. However, instead of causing the disease, the genetic material contained in the viral vector is modified to replace or alter the flawed gene or to introduce therapeutic genes to treat the disease. A viral vector is the most efficacious method of gene transfer to modify the host cell and introduce a therapeutic gene. Viral vectors are efficient at nucleic acid delivery and also avoid immunosurveillance by the host. Several various types of viruses have been altered for the use of gene therapy including retrovirus, herpes simplex virus, adeno associated virus (AAV) and adenovirus.

Viral vectors are modified in laboratories to become suitable for gene therapy as each of them have different advantages and disadvantages. Retroviral vectors need mitotic cell division for transduction, but they can permanently integrate into the genome of the infected cell. Gene delivery by adenoviral vectors is effective for a broad range of dividing and nondividing cell types; nevertheless, the expression of genes in vivo is frequently restricted due to immune clearance of infected cells. The herpes simplex virus can transfer significant amounts of foreign DNA, yet it still faces challenges with cytotoxicity and transgenic expression maintenance. AAV has a limited capacity for DNA, yet it can infect a wide variety of dividing and nondividing cell types.

AAV belongs to genus Dependoparvovirus, belonging to the family Parvoviridae. Three genes, *Rep*, *Cap*, and *aap*, are found in its single-stranded genome. By using three promoters, alternative translation start sites, and variable splicing, these three genes produce at least nine distinct gene products. Inverted terminal repeats (ITRs), which are necessary for genome packing and replication, are positioned on either side of these coding regions. While the expression of the *Cap* gene results in the production of the viral capsid proteins which form the outer capsid shell that protects the viral genome and are actively involved in cell binding and internalisation, the *Rep* gene encodes four proteins (Rep78, Rep68, Rep52, and Rep40) that are necessary for viral genome replication and packaging.

The AAV2 viral vector is used for PD gene therapy because of its nonimmunogenic, non-integrating, nonpathogenic and stable properties. The therapy contains a mixture of two AAV vectors encoding GAD-65 and GAD-67 enzyme isoforms. AAV2 carries the GAD (glutamic acid carboxylase) gene which codes for increased production of GABA (gamma aminobutyric acid), which is an inhibitory neurotransmitter, to the subthalamic nucleus (STN). The subthalamic nucleus in the brain is a vital part of the basal ganglia that regulates undesirable movements and helps in motor control. It contains approximately 5,50,000 neurons connecting across the cortico-basal ganglia- thalamo- cortical network (CBGTC loop) and also prevents unwanted movements by increasing production of the neurotransmitter glutamate (2-Aminopentanedioic acid) to the internal globus pallidus. Patients suffering from PD face the effects of increased activity of the subthalamic nucleus due to the loss of inhibitory neurons which manifest into physical symptoms such as tremors, bradykinesia, akinesia, muscle rigidity etc.

AAV2-GAD gene therapy aims to increase levels of GABA and regulate neural activity in the subthalamic nucleus. AAV2 serves as the delivery system for the functional gene. After a surgical operation, GAD is infused directly into the subthalamic nucleus, where it is released from its delivery system and into neurons. The GAD gene codes for the creation of an enzyme known by the same name, GAD, which is essential for the synthesis of GABA, a signalling molecule that regulates excessive brain activity. GABA levels are predicted to rise in the subthalamic nucleus by enabling neurons there to produce GAD on their own. Thus, it is anticipated that this will protect the signalling pathways in charge of regulating movement, thus mitigating Parkinson's disease symptoms.

Researchers insert the GAD gene into the AAV viral vector and it is surgically delivered to the STN bilaterally through catheter infusion. This is a stereotactic surgery where a three dimensional coordinate system is used to obtain the targeted area of the brain. A burr hole created during trepanning is made in the skull and a thin catheter guides the surgeons to the STN through imaging guidance. Once arrived at the target area, the AAV-GAD vector is successfully injected.

A study was conducted at the New York Presbyterian Hospital where 12 PD patients were injected with AAV2-GAD viral vectors. Four were injected with a low dose, four with a medium dose and four with a high dose. Each patient suffered stage 3 in the Hoehn and Yahr scale, symptoms being- mild to moderate

bilateral involvement and slight postural instability but physically independent. Progress was recorded and evaluated regularly after 1, 3, 6 and 12 months at the North Shore Hospital through PET scans with 18F fluorodeoxyglucose, Unified Parkinson's Disease Rating Scale (UPDRS), neuropsychological testing and scales of activities of daily life.

Three months following gene therapy, there were significant improvements in motor UPDRS scores (p=0.0015), primarily on the side of the body that was contralateral to surgery. These benefits lasted for up to a year. PET scans showed a significant decrease in brain metabolism in the supplementary motor area of the brain, which was limited to the treated hemisphere, as well as a relationship between clinical motor scores and brain metabolism.

AAV-GAD gene therapy was declared as a safe procedure with a positive response from the patients, suggesting that in-vivo gene therapy was suitable for patients with neurodegenerative ailments.

## CONCLUSION

AAV2-GAD gene therapy proves as a successful in-vivo gene therapy treatment for advanced PD patients. It is minimally invasive as compared to other surgical procedures to treat PD, like deep brain stimulation (DBS). AAV vectors can alter long term gene expression leading to prolonged benefits and improvement in motor skills.

Researchers are currently working on advancements in this procedure. Future prospects seem positive and viable which include optimised delivery methods, technological advancements and enhanced vector designs. Researchers are exploring the possibilities of merging CRISPR Cas9 genome editing with AAV vectors to find a definite cure for PD and alter underlying genetic mutations to prevent further inheritance of the disease. Exploring methods of non-invasive procedures such as intranasal delivery or focused ultrasound are also being researched about.

AAV gene therapy shows promising results and has a high scope for treating neurodegenerative diseases. Owing to its simple structure, rare biological composition and conducive properties, AAV possesses the possibility of becoming the most suitable vector for most genetic therapies. Future regulatory approvals and improvements to patient outcomes and health will undoubtedly come from better designed trials, optimised vector construction, and innovative AAV variations.

## CITATION

1. NHS. "Parkinson's Disease - Treatment." *Nhs.uk*, 23 Oct. 2017, www.nhs.uk/conditions/parkinsons-disease/treatment/#:~:text=Most%20people%20with%20Parkinson.
2. "The Genetic Link to Parkinson's Disease." *Www.hopkinsmedicine.org*, 10 Apr. 2022, www.hopkinsmedicine.org/health/conditions-and-diseases/parkinsons-disease/the-genetic-link-to-parkinsons-disease#:~:text=Genes%20Linked%20to%20Parkinson.
3. "Parkinson's Disease: Causes, Symptoms, and Treatments." *National Institute on Aging*, 14 Apr. 2022, www.nia.nih.gov/health/parkinsons-disease/parkinsons-disease-causes-symptoms-and-treatments#symptoms.
4. Axelsen, Tobias M., and David P.D. Woldbye. "Gene Therapy for Parkinson's Disease, an Update." *Journal of Parkinson's Disease*, vol. 8, no. 2, 13 June 2018, pp. 195–215, www.ncbi.nlm.nih.gov/pmc/articles/PMC6027861/, https://doi.org/10.3233/jpd-181331.
5. Oczkowska, Anna, et al. "Mutations in PRKN and SNCA Genes Important for the Progress of Parkinson's Disease." *Current Genomics*, vol. 14, no. 8, Feb. 2014, pp. 502–17,

https://doi.org/10.2174/1389202914666131210205839.
6. Polymeropoulos, M. H., et al. "Mutation in the Alpha-Synuclein Gene Identified in Families with Parkinson's Disease." *Science (New York, N.Y.)*, vol. 276, no. 5321, 1997, pp. 2045–47, https://doi.org/10.1126/science.276.5321.2045.
7. De Felipe, Pablo, and Marta Izquierdo. "Tricistronic and Tetracistronic Retroviral Vectors for Gene Transfer." *Human Gene Therapy*, vol. 11, no. 13, Sept. 2000, pp. 1921–31, https://doi.org/10.1089/10430340050129530.
8. Wang, William W., et al. "The G209A Mutation in the α-Synuclein Gene Is Not Detected in Familial Cases of Parkinson Disease in Non-Greek And/or Italian Populations." *Archives of Neurology*, vol. 55, no. 12, Dec. 1998, p. 1521, https://doi.org/10.1001/archneur.55.12.1521. Accessed 4 May 2020.
9. Tagliafierro, L., and O. Chiba-Falek. "Up-Regulation of SNCA Gene Expression: Implications to Synucleinopathies." *Neurogenetics*, vol. 17, no. 3, 7 Mar. 2016, pp. 145–157, https://doi.org/10.1007/s10048-016-0478-0.
10. Mata, Ignacio F., et al. "SNCA Variant Associated with Parkinson Disease and Plasma α-Synuclein Level." *Archives of Neurology*, vol. 67, no. 11, 1 Nov. 2010, https://doi.org/10.1001/archneurol.2010.279.
11. Siddiqui, Irum Javaid, et al. "The Parkinson Disease Gene SNCA: Evolutionary and Structural Insights with Pathological Implication." *Scientific Reports*, vol. 6, no. 1, 15 Apr. 2016, www.ncbi.nlm.nih.gov/pmc/articles/PMC4832246/, https://doi.org/10.1038/srep24475.
12. Wang, William W., et al. "The G209A Mutation in the α-Synuclein Gene Is Not Detected in Familial Cases of Parkinson Disease in Non-Greek And/or Italian Populations." *Archives of Neurology*, vol. 55, no. 12, 1 Dec. 1998, p. 1521, https://doi.org/10.1001/archneur.55.12.1521.
13. Sidransky, Ellen, and Grisel Lopez. "The Link between the GBA Gene and Parkinsonism." *The Lancet Neurology*, vol. 11, no. 11, Nov. 2012, pp. 986–998, www.ncbi.nlm.nih.gov/pmc/articles/PMC4141416/, https://doi.org/10.1016/s1474-4422(12)70190-4.
14. Barbuti, Peter A, et al. "Gene-Corrected P.A30P SNCA Patient-Derived Isogenic Neurons Rescue Neuronal Branching and Function." *Scientific Reports*, vol. 11, no. 1, 9 Nov. 2021, https://doi.org/10.1038/s41598-021-01505-x.
15. LeWitt, Peter A, et al. "AAV2-GAD Gene Therapy for Advanced Parkinson's Disease: A Double-Blind, Sham-Surgery Controlled, Randomised Trial." *The Lancet Neurology*, vol. 10, no. 4, Apr. 2011, pp. 309–319, https://doi.org/10.1016/s1474-4422(11)70039-4.
16. OpenAI. "ChatGPT ." *ChatGPT*, 2024, chatgpt.com/.
17. Dumbhare, Omkar, and Sagar S Gaurkar. "A Review of Genetic and Gene Therapy for Parkinson's Disease." *Cureus*, vol. 15, no. 2, 5 Feb. 2023, https://doi.org/10.7759/cureus.34657.
18. Li, J, et al. "Effect of Familial Parkinson's Disease Point Mutations A30P and A53T on the Structural Properties, Aggregation, and Fibrillation of Human Alpha-Synuclein." *Biochemistry*, vol. 40, no. 38, 2001, pp. 11604–13, www.ncbi.nlm.nih.gov/pubmed/11560511, https://doi.org/10.1021/bi010616g.
19. Lu, Jianqing, et al. "Comparison between α-Synuclein Wild-Type and A53T Mutation in a Progressive Parkinson's Disease Model." *Biochemical and Biophysical Research Communications*, vol. 464, no. 4, Sept. 2015, pp. 988–993, www.sciencedirect.com/science/article/pii/S0006291X15302382, https://doi.org/10.1016/j.bbrc.2015.07.007. Accessed 17 June 2019.
20. Zhang, Xue-Ming, et al. "The A30P α-Synuclein Mutation Decreases Subventricular Zone Proliferation." *Human Molecular Genetics*, vol. 28, no. 14, 19 Mar. 2019, pp. 2283–2294,

https://doi.org/10.1093/hmg/ddz057.
21. Zarranz, Juan J., et al. "The New Mutation, E46K, of α-Synuclein Causes Parkinson and Lewy Body Dementia." *Annals of Neurology*, vol. 55, no. 2, 30 Dec. 2003, pp. 164–173, https://doi.org/10.1002/ana.10795.
22. Zhao, Kun, et al. "Parkinson's Disease Associated Mutation E46K of α-Synuclein Triggers the Formation of a Distinct Fibril Structure." *Nature Communications*, vol. 11, no. 1, 26 May 2020, https://doi.org/10.1038/s41467-020-16386-3.
23. Boyer, David R., et al. "The α-Synuclein Hereditary Mutation E46K Unlocks a More Stable, Pathogenic Fibril Structure." *Proceedings of the National Academy of Sciences*, vol. 117, no. 7, 3 Feb. 2020, pp. 3592–3602, https://doi.org/10.1073/pnas.1917914117.
24. Flagmeier, Patrick, et al. "Mutations Associated with Familial Parkinson's Disease Alter the Initiation and Amplification Steps of α-Synuclein Aggregation." *Proceedings of the National Academy of Sciences*, vol. 113, no. 37, 29 Aug. 2016, pp. 10328–10333, www.ncbi.nlm.nih.gov/pmc/articles/PMC5027465/#r14, https://doi.org/10.1073/pnas.1604645113.
25. "ClinicalTrials.gov." *Clinicaltrials.gov*, clinicaltrials.gov/study/NCT00195143.
26. Luo, Jia, et al. "Subthalamic GAD Gene Therapy in a Parkinson's Disease Rat Model." *Science (New York, N.Y.)*, vol. 298, no. 5592, 11 Oct. 2002, pp. 425–429, pubmed.ncbi.nlm.nih.gov/12376704/, https://doi.org/10.1126/science.1074549.
27. During, M. J., et al. "Subthalamic GAD Gene Transfer in Parkinson Disease Patients Who Are Candidates for Deep Brain Stimulation." *Human Gene Therapy*, vol. 12, no. 12, 10 Aug. 2001, pp. 1589–1591, pubmed.ncbi.nlm.nih.gov/11529246/. Accessed 27 June 2024.
28. Muramatsu, Shin-ichi. "The Current Status of Gene Therapy for Parkinson's Disease." *Annals of Neurosciences*, vol. 17, no. 2, 1 Apr. 2010, pp. 92–95, www.ncbi.nlm.nih.gov/pmc/articles/PMC4116997/, https://doi.org/10.5214/ans.0972-7531.1017209.
29. Pd, Robbins, et al. "Viral Vectors for Gene Therapy." *Trends in Biotechnology*, 1 Jan. 1998, pubmed.ncbi.nlm.nih.gov/9470229/.
30. Ligaard, Johanne, et al. "Deep Brain Stimulation and Genetic Variability in Parkinson's Disease: A Review of the Literature." *Npj Parkinson's Disease*, vol. 5, no. 1, 6 Sept. 2019, https://doi.org/10.1038/s41531-019-0091-7.
31. Kingwell, Katie. "Gene Therapy for Parkinson Disease Shows Promise in Phase II Trial." *Nature Reviews Neurology*, vol. 7, no. 5, 12 Apr. 2011, pp. 241–241, https://doi.org/10.1038/nrneurol.2011.48.
32. Fuchs, Julia, et al. "Genetic Variability in TheSNCAgene Influences α-Synuclein Levels in the Blood and Brain." *The FASEB Journal*, vol. 22, no. 5, May 2008, pp. 1327–1334, https://doi.org/10.1096/fj.07-9348com.
33. *Springernature.com*, 2024, media.springernature.com/lw685/ springer-static/image/art%3A10.1186%2Fs40478-023-01687-7/MediaObjects/40478_2023_1687_Fig6_HTML.png.
34. Kouli, Antonina, et al. "Table 1, PARK-Designated Genes Involved in Familial Parkinson's Disease." Www.ncbi.nlm.nih.gov, 21 Dec. 2018, www.ncbi.nlm.nih.gov/books/NBK536722/table/Ch1-t0001/.
35. https://molecularneurodegeneration.biomedcentral.com/articles/10.1186/s13024-023-00675-8/figures/1
36. https://www.researchgate.net/figure/Chromosomal-location-of-SNCA-gene-41_fig1_330949083