# Shedding light on gene therapy of Parkinson's disease in non-human primates

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Parkinson's disease (PD) is a progressive neurodegeneration disease initially described by James Parkinson. It is typically diagnosed based on clinical features such as bradykinesia, resting tremor, rigidity, and, in later stages, postural instability. Epidemiologically, the prevalence of PD ranges from 5 to over 35 cases per 100,000 population, with the majority affecting individuals aged 50 years or older. The risk increases with age, particularly in males. Various factors, including genetics, lifestyle, specific environmental influences, and geometry, contribute to the disease.[1] The primary cause of PD is the loss of nigrostriatal dopaminergic innervation due to neurodegeneration affecting neurons in the substantia nigra pars compacta (SNc). Underlying pathological mechanisms involve aggregated α-synuclein, mitochondrial dysfunction, oxidative stress, and neuroinflammation.[1] Regardless of the specific mechanism, the manifestation of motor dysfunction or cognitive deficits in PD occurs when basal ganglion (BG) neural networks fail to function normally due to dopamine depletion.

Dopamine is a crucial neural modulator, primarily governing the activity of spiny projection neurons (SPNs) located in the striatum, the principal input structure of the BG. Two distinct subtypes of SPNs exist: the D1-receptor-expressing direct pathway neurons (D1-SPNs), which project axons to BG output regions, including the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr), and the D2-receptor-expressing indirect pathway neurons (D2-SPNs), which regulate BG output through a multisynaptic pathway. Empirical evidence suggests that dopamine presence suppresses the activity of D2-SPNs while facilitating firing in D1-SPNs under normal physiological conditions. An imbalance in the activity of D1- and D2-SPNs emerges following dopamine depletion, leading to heightened thalamic inhibition. This imbalance is believed to be the primary contributing factor to the motor symptoms observed in PD.[1]

The predominant therapeutic intervention for PD in clinical settings is the supplementation of levodopa, which is crucial for dopamine synthesis. Nonetheless, this approach relies on the presence of residual dopaminergic neurons and is associated with side effects, including off-target effects, motor fluctuations, and dyskinesias. In certain instances, healthcare professionals prescribe dopamine receptor agonists. For individuals in advanced stages where conventional drug therapy proves ineffective, the consideration of deep brain stimulation (DBS) follows a thorough evaluation.[1] While DBS has demonstrated a notable improvement in motor scores, ranging from 50% to 60%, it necessitates invasive surgical procedures and enduring implantation of electrodes within patients' brains, presenting challenges related to infection and electrode quality.[1] A novel treatment paradigm aims to functionally restore the dopaminergic network by implanting stem cells within the dopaminergic system.[1] However, the effectiveness and safety of this approach still require further validation.

A novel research avenue explores a circuit-based approach to reinstate the functionality of the BG in PD. In a recently published study in the journal *Cell*,[2] Chen, Hong, and their colleagues pioneered an innovative genetic strategy designed to label and manipulate striatal D1-SPNs in both mice and non-human primates. This innovative approach holds promise for future clinical applications in PD.

The genesis of this innovative approach was grounded in the hypothesis that augmenting the excitability of the direct pathway in PD could help alleviate motor deficits. In a preceding study involving mice, optogenetic activation of D1-SPNs in the dorsal striatum increased locomotion in normal mice and significantly ameliorated motor deficits in a 6-OHDA-induced PD model.[3] While transgenic lines are commonly employed in mouse research, their feasibility in non-human primate studies, let alone their clinical translation for patient application, remains

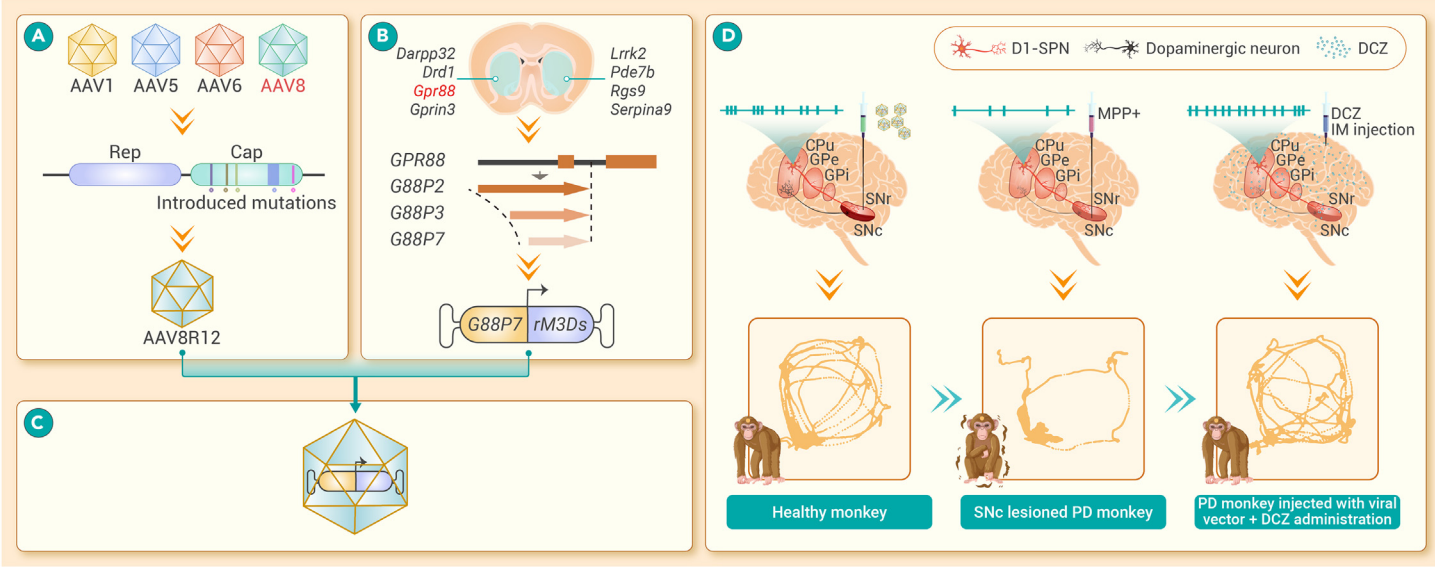


**Figure 1. The development of a new circuit-based gene therapy for Parkinson's disease** (A) Modification of the capsid protein of AAV8 results in the production of retrograde AAV8R12. (B) New promoters, specifically *G88P3* and *G88P7*, are derived from *G88P2*—the promoter originating from *GPR88*, a gene notably enriched in the striatum. (C) Combining the newly developed AAV8R12 virus with the initiation of gene expression from the new promoter results in an efficient viral vector capable of retrograde labeling striatum SPNs. (D) The retrograde viral vector is injected into the SNr of the PD monkey model, labeling the direct pathway D1-SPNs with the DREADD effector. Additional administration of deschloroclozapine (DCZ) increased the activity of D1-SPNs after dopaminergic neuron lesion induced by MPP+. This approach successfully reverses the core motor deficits observed in the PD monkey.

constrained. In navigating this challenge, the research team leveraged the specificity of D1-SPN projection to the SNr (Figure 1), employing a retrograde-virus-based gene delivery system.[2]

The primary challenge encountered was the limited effectiveness of virus infection. In addressing this, the researchers developed a novel adeno-associated virus (AAV), AAV8R12, derived from AAV8 and specifically engineered to heighten retrograde infectivity (Figure 1A). Notably, this innovative virus demonstrated higher efficiency in labeling D1-SPNs when administered within the SNr. Furthermore, the research team identified and adapted a promoter, *G88P2*, originating from the *GPR88* gene (Figure 1B), which exhibited superior expression levels in SPNs compared to commonly employed promoters like *hSyn*, *EF1a*, and *CAG*. They also optimized the promoter to enhance the tool's effectiveness. As a result, approximately one-fifth of D1-SPNs in the striatum, or the caudate putamen (CPu), of non-human primates were successfully labeled.

Utilizing this potent gene delivery system (Figure 1C), the researchers successfully expressed a chemogenetic DREADD effector in the D1-SPNs of both mice and non-human primates. Their meticulous strategy, involving the co-expression of both Cre and rM3Ds in D1-SPNs, coupled with the injection of a Cre-dependent ChR2 virus into the CPu, facilitated *in vivo* opto-tagging of D1-SPNs. In this way, they confirmed that the administration of the rM3Ds ligand clozapine N-oxide (CNO) specifically facilitated the firing of D1-SPNs. Subsequent behavioral tests provided evidence demonstrating rapid and enduring effects upon systemic administration of ligands, CNO, or deschloroclozapine. This treatment induced a notable increase of local motion in healthy animals and, significantly, the reversal of core disease phenotypes in the animal models of PD (Figure 1D). Notably, the chemogenetic manipulation exhibited remarkable durability, retaining its effectiveness even months after the initial viral injection. Collectively, these attributes signify a promising advancement in the potential treatment of PD.

This work holds substantial value for several reasons. Firstly, the researchers introduced a transgenic tool and conducted comprehensive evaluations in non-human primates. AAV, widely employed and considered safe in gene therapy, has faced challenges in primate research and clinical settings, often displaying varied efficacy compared to mouse models. The comprehensive assessments conducted in both mice and non-human primates in this study represent a significant step toward clinical translation given the greater biological similarity between non-human primates and humans than mice.

Secondly, while optogenetic activation of D1-SPNs in the dorsal striatum of mice has shown promise in improving motor deficits, manipulating D1-SPNs labeled throughout the entire CPu in primates presents logistical challenges with light delivery through a small tip. Additionally, optogenetic manipulation necessitates opto-fiber implantation, carrying the potential for immunological rejection. In this regard, chemogenetic manipulation, administering DREADD ligands, enables the activation of all labeled neurons without the need for implantation, effectively overcoming these challenges.

Thirdly, this method primarily targets the direct pathway, which is highly selective in neural circuits. Compared to levodopa treatment, it has shown reduced side effects and a prolonged effect lasting 24 h after ligand administration, surpassing the limited 6 h window of levodopa. Moreover, this technique involves a single, straightforward surgical procedure for viral vector delivery, with regular ligand administration, eliminating the need for electrode implantation as required in DBS. This characteristic enhances its safety in application.

Despite these advancements, significant challenges remain before clinical application. Prior gene therapy attempts, introducing glial cell line-derived neurotrophic factor (GDNF) or dopamine synthesis enzymes into the striatum, aimed to reconstruct the dopamine terminal or induce local dopamine release.[1] GDNF's efficacy relies on residual dopaminergic terminals, limiting its effectiveness to early disease stages. Local dopamine synthesis, feasible in animal studies, raises concerns about side effects akin to levodopa administration. The current approach mandates precise viral injection into the SNr—a relatively small nucleus nestled deep within the brain—without any unintended leakage. Otherwise, with the potent retrograde labeling capacity of the virus, the chemogenetic manipulation may inadvertently activate undesired brain areas, resulting in unknown effects. While significant progress has been made, the pursuit of the ideal tool for PD remains an intricate and ongoing endeavor.

A growing body of evidence highlights that the direct and indirect pathways within the BG function in a complementary manner, alongside their role in counterbalancing.[4] Furthermore, it is crucial to acknowledge that, beyond the striatum, dopamine dysregulation systematically impacts the entire BG and beyond in PD. Research has revealed that dopamine application activates neurons in the globus pallidus externus (GPe) and the subthalamic nucleus (STN), both integral components of the downstream indirect pathway. The excitatory STN-GPe and inhibitory GPe-STN projections form a reciprocally connected loop, contributing to oscillatory activity. In parkinsonian animals, the activity in GPe and STN neurons becomes synchronous and correlated.[5] Notably, STN is one of the major targets for DBS in the treatment of PD. However, the intricate neural circuit mechanisms governing BG function, particularly concerning these pathways, are still not fully understood. This complexity adds a layer of challenge to the quest for a comprehensive treatment.

Patients with PD typically share a common diagnosis of dopaminergic system deficits and varying degrees of motor deficits, yet their symptom profiles often exhibit unique characteristics. While the systematic restoration of direct pathway activity is beneficial, it may only offer partial relief from these symptoms. Additionally, the evaluation conducted by the authors predominantly relied on general movement observations and subjective PD scoring, representing a promising start. Nevertheless, it would be highly valuable to explore the potential for more comprehensive behavioral tests. Such tests would provide a deeper understanding of the intervention's impact on both motor and non-motor function, thereby enhancing our ability to develop effective treatments for PD. Therefore, the continued pursuit of improvement and rigorous evaluation of these interventions remains essential in the ongoing quest for more effective PD treatment.

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## DECLARATION OF INTERESTS

The authors declare no competing interests.