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Gene therapy for Parkinson's Disease and Ethical Challenges: A Systematic Review

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Abstract

Background: Parkinson's disease (PD) is a complex, multifactorial neurodegenerative disorder with a pathophysiology deriving from the synergy of abnormal aggregation of neuroinflammation, synuclein and dysfunction of lysosomes, mitochondria and synaptic transport difficulties influenced by genetic and idiopathic factors. Worldwide, PD has a prevalence of 2-3% in people over the age of 65. To date, there is no certified, effective treatment for PD. **Aim:** The aims of this research were: (i) to present, on the basis of recent advances in molecular genetics and epigenetics, the genomic aspects and challenges of gene therapy trials for PD; (ii) to outline the ethical principles applicable to therapeutic trials for PD. **Method:** A systematic literature review was carried out to identify relevant articles reporting on genomic aspects and gene therapy in PD from 2001 to October 2023. The search was conducted in French and/or English in three databases: PubMed, Google Scholar and Science Direct. PRISMA guidelines were used in this systematic review. **Results:** A total of thirty-three publications were selected. An inductive thematic analysis revealed that numerous genetic mutations (SNCA, Parkin, PINK1, DJ-1, LRRK2, ATP13A2, VPS35, Parkin/PRKN, PINK1, DJ1/PARK7) and epigenetic events such as the action of certain miRNAs (miR-7, miR-153, miR-133b, miR-124, miR-137) are responsible for the onset of PD, and that

genetic therapy for this pathology raises ethical questions that need to be elucidated in the light of the bioethical principles of autonomy, beneficence, non-maleficence and justice. **Conclusion:** There is no zero risk in biotechnology. Then, it will be necessary to assess all the potential risks of Parkinson disease's gene therapy to make the right decision. It is therefore essential to pursue research and, with the guidance of ethics, to advance treatment options and meet the challenges of brain manipulation and its impact on human identity. The golden rule of medicine remains: "*Primum non nocere*".

Keywords

Neurodegenerative Diseases, Parkinson Disease, Molecular Mechanism, Gene Therapy, Gene Therapy Ethics

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease in the world [1] [2]. It is characterized by the progressive loss of dopaminergic neurons (DN) in the substantia nigra pars compacta (SNPC), leading to a significant decrease in dopamine levels reaching the striatum and subsequent functional impairment of the motor circuit, resulting in parkinsonism [3]. PD, whose etiology is made up of motor symptoms (bradykinesia, slow movements, involuntary movements, tremors, gait disorders, imbalance and rigidity) and non-motor symptoms (cognitive disorders, mental health disorders, sleep disorders, sensory disorders, dementia, depression and anxiety), is a disabling pathology [4]. PD has a prevalence of more than 1% in people aged 60 to 64 years and 2% to 3% in people older than 65 years [5]. PD can be sporadic or familial, the latter accounting for 10% to 15% of the total [6]. The familial type is generally caused by mutations in genes including SNCA, Parkin, PINK1, DJ-1, LRRK2 and ATP13A2 linked to Parkinson's disease. Thus, mutations in at least six genes are linked to the pathogenesis of PD: the LRRK2, SNCA and VPS35 genes for dominant forms, and the Parkin/PRKN, PINK1 and DJ1/PARK7 genes for recessive forms [7]. On the other hand, other neurodegenerative diseases such as Alzheimer's disease (AD), Huntington's disease (HD), Lewy body dementia (LBD), fronto-temporal dementia (FTD), amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) are associated with changes in nerve cells and brain tissue. These neurodegenerative pathologies, as their name suggests, cause the progressive degeneration of nerve cells in the brain, and have a major impact on the functional capacities of the people they affect. They generally induce movement, thought (cognitive) and even psychiatric disorders in these individuals. Alzheimer's disease, for example, is caused by a number of genetic mutations and epigenetic regulations. (PSEN1; PSEN2; APP; APOE ε4; TREM2; SORL1; ABCA7; CR1; DAPK1; CLU; PICALM; BIN1; MS4A6A; CD33; CD2AP; EPHA1; miR-124; miR-15 et miR-146a) [8] and Lewy body dementia (LBD) (GBA, SNCA, APOE, CNTN1, BCL7C/STX1B,

SCARB2 [9], miR-24-3p, miR-25-3p, miR-3p, miR-361-5p, miR-425x5p, et miR-451a [10]. Whereas Huntington's disease is caused by an expansion of the polymorphic CAG trinucleotide by more than 35 repeats in exon 1 of the HTT gene [11]. In addition, the actions of miR-124, MIR-132 and MIR-9 contribute to neurodegeneration. [12]. Based on the therapeutic objectives of Parkinson's disease, four categories of therapeutic approach are currently being developed [13]. The first strategy is to boost the bioavailability of brain DNA in order to stimulate brain regeneration; the second technique relies on neurotrophic factors and neuromodulation in the subthalamic nucleus (STN); the third strategy focuses on genes involved in the mitochondrial pathway and mitophagy; finally, the fourth technique involves decreasing z-synuclein synthesis, which helps to attenuate the effects of altered mitochondrial pathways [14].

Gene therapy opens up great therapeutic prospects for genetic diseases. But what are the treatment options after their presymptomatic diagnosis? Gene therapy for PD and all other neurodegenerative diseases raises ethical questions that need to be considered in the light of the fundamental principles of bioethics. Like other neurodegenerative diseases, the requirements of ethical standards in PD gene therapy are based not only on autonomy, beneficence and non-maleficence, but also on the principle of precaution, respect for human dignity, confidentiality and the free and informed consent of the patient involved. The objectives of this research were to present the genomic aspects and challenges of gene therapy trials for PD, drawing on recent advances in molecular genetics, epigenetics and the ethical principles applicable to therapeutic trials for these pathologies.

2. Methodology

We carried out a systematic review, using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.

2.1. Data Sources

A systematic literature review was carried out to identify relevant articles reporting on genomic aspects and gene therapy of Parkinson's disease from 2001 to October 2023. The search was conducted in French and/or English in three databases: PubMed, Google Scholar and Science Direct.

2.2. Study Selection

Studies were included if they reported on 1) genomic and epigenetic aspects, 2) clinical and physiological aspects, 3) gene therapy for Parkinson's disease and 4) the ethical issue. The keywords used were "neurodegenerative diseases", "Parkinson's disease", "gene therapy for MND" and "bioethical principles". Two reviewers (TMZ and JS) independently identified articles and reviewed them sequentially (titles, abstracts, then full texts) for inclusion (**Figure 1**). For articles without an abstract, or without sufficient information in the abstract to make a decision, the full text and, if necessary, supplementary material were reviewed

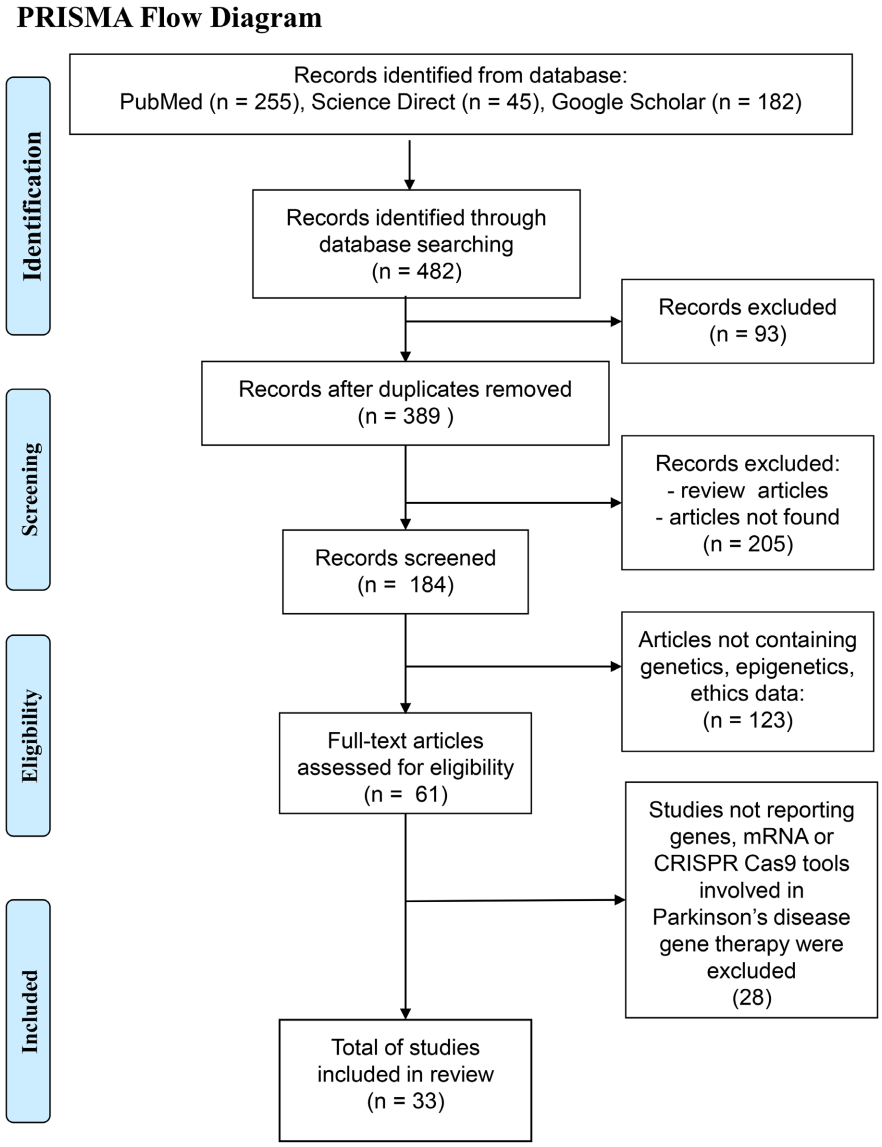

Figure 1. Flow diagram showing the method for the study selection. The database search for the search strategy described in the section was cleaned up to exclude review articles and duplicates. Titles and abstracts were included in the literature review. Review articles, articles with ambiguous data that did not meet the inclusion criteria were then excluded during the full-text review thirty-three (33) relevant articles were finally included for this review.

before a decision was made. Duplicate publications, review articles, studies not reporting gene information, miRNA or CRISPR/Cas9 tools involved in gene therapy for Parkinson's disease, research conducted excluding pediatric populations and studies conducted on migrant populations were excluded. **Figure 1** shows the study selection process.

2.3. Data Extraction

Two reviewers (TMZ and JS) independently performed data extraction from the included studies. Extracted data covered study contexts, design, population cha-

racteristics, measures of disease onset (prevalence), genomic and epigenetic aspects, pathophysiological aspects, therapeutic and ethical issues for the different conditions examined. Data were exported to Endnote X8 software and duplicates were removed. The next stage in the selection process involved a thorough review of the abstracts of eligible articles. Studies without abstracts or with irrelevant information were then excluded. The final selection of studies included in this review was made through a comprehensive evaluation of the full texts.

2.4. Data Analysis

A detailed inductive thematic analysis and narrative synthesis of the full texts has been carried out, and these are then presented under key sub-themes relevant to this systematic review.

3. Results and Discussion

3.1. Articles Reviewed and Included in the Study

Figure 1 shows a flow chart of the selection process for studies included in the PRISMA systematic review (Figure 1).

3.2. Genetic Aspects of Parkinson's Disease

Parkinson's disease (PD) is a complex, multifactorial neurodegenerative disorder. Various risk factors are involved in the pathogenesis of PD. Both inherited and acquired genetic mutations lead to the generation of abnormal proteins. Over the last few decades, considerable advances have been made in genomic science to understand the genetic and epigenetic basis of Parkinson's disease (PD), with the discovery of 17 Mendelian inherited genes, including 6 autosomal dominant (AD), 10 autosomal recessive (AR) and 1 X-linked gene, and almost one hundred and fifty (150) localized genetic susceptibility factors [15]. Thus, the difficulties in deciphering the pathogenic mechanisms of PD are due, to a large extent, to the multiplicity of Mendelian-inherited mutations PD (SNCA, LRRK2, VPS35, HTRA2, EIF4G1, GBA or AR (PARK2 (encoding Parkin protein), PINK1, PARK7 (known as DJ-1), ATP13A2, PLA2G6, FBXO7) [16]. Thus, various genes are incriminated as monogenic causes of PD (mutations in SNCA (or PARK1) and LRRK2 (or PARK8) are associated with autosomal dominant transmission of PD), while parkinsonism-related disorders are caused by mutations in PRKN (or PARK2), PINK1 (or PARK6) and DJ-1 (or PARK7), which are responsible for autosomal recessive inherited forms [17]. As for the SNCA gene encoding α-synuclein, the molecular processes by which its mutations lead to PD are thought to be a gain-of-function mechanism, capable of inducing aberrant aggregation of the α-Syn protein and, consequently, leading to cell damage and neuronal death. In addition, mutations in the Leucine-Rich Repeat Kinase 2 (LRRK2 or PARK8) gene are among the main causes of familial Parkinson's disease. It should be noted that all these mutations confer an increased risk of developing PD, even if, in some cases, their effect is modest due to their low

penetration. Many mutations have been shown to up-regulate kinase activity and increase LRRK2 autophosphorylation. Indeed, according to West *et al.* 2005 [18], the G2019S mutation is the most common and penetrating mutation in LRRK2, and is also the single-factor cause of the most common PD identified. It is believed to increase LRRK2 autophosphorylation in a pathogenic gain-of-function mechanism. Other new genes have reportedly been recognized for their contribution to monogenic dominant PD: Glucocerebrosidase (GBA), which codes for a lysosomal protein that degrades glucocerebroside, and Vacuolar Sorting Protein 35 (VPS35), CCHHD2, TMEM230 and RIC3 [17]. The autosomal recessive Parkin gene (PRKN or PARK2) causes juvenile forms of early-onset PD. Parkin is an E3-type ubiquitin ligase dedicated to the degradation of α-Syn and other substrates. According to Goldber *et al.* 2003 [19], the PRKN gene, which is mutated, has catabolized the degradation of α-Syn hence its accumulation subsequently leads to the selective death of neurons in the substantia nigra. Furthermore, according to Rauschendorf *et al.*, 2017 [20], mutations in the PTEN 1-induced kinase gene (PINK1 or PARK6) also cause features similar to those due to parkin mutations, with typical early motor symptoms, slow progression and absence of non-motor symptoms. The Daisuke Junko-1 (DJ-1 or PARK7) gene encodes a transcriptional regulator that protects mitochondria from oxidative stress by enhancing the expression of two mitochondrial uncoupling proteins (UCP4 and UCP5), thereby lowering mitochondrial membrane potential and suppressing ROS production, optimizing a number of mitochondrial functions and promoting neuronal cell survival [21]. Today, mitochondrial dysfunction, oxidative stress, altered protein processing and inflammatory changes have been suggested as causes of neuronal dysfunction and death by apoptosis or autophagy. **Table 1** shows the genes implicated in Parkinson's disease, which could potentially be targeted for gene therapy. With this in mind, gene-editing tools such as transcription activator-like effector nucleases (TALENs) and CRISPR Cas9 are now being used in therapeutic trials. These technologies can edit, replace and modify defective sites on the genome for gene therapy of neurodegenerative disorders such as Alzheimer's disease, Huntington's disease and Parkinson's disease.

3.3. Gene Therapy for Parkinson's Disease

To this day, there are no effective medications for PD and palliative treatments, such as levodopa administration, only aim to relieve motor symptoms but induce serious side effects over time [22]. There is therefore an urgent need to discover new drugs in order to design more effective therapeutic approaches. The identification of mutations and polymorphisms in autosomal dominant and recessive genes, and of epigenetic alterations such as the deregulation of various miRNAs that may stimulate many aspects of Parkinson's disease pathogenesis, has opened up a dynamic new scenario in the search for effective new treatments. Gene therapy involves introducing a transgene or modified endogenous

Table 1. Genes implicated in Parkinson's disease and potentially targeted for gene therapy.

Authors	Gene	Fonction of the gene	Consequences of genetic mutation
Elnageeb *et al.*, 2023 [43]	SNCA/PARC 1/ PARC 4	Regulator of dopamine biosynthesis, release and transport; apoptosis suppressor.	The widespread presence of Lewy bodies throughout the brain and cerebral cortex, as well as neuronal destruction in the locus coeruleus (LC) and SN.
Zimprich *et al.*, 2004 [44]	LRRK2/PARC8	The Roco protein family includes the LRRK2 gene component. It is involved in cytoskeletal dynamics, autophagy and vesicular transport	LRRK2 mutations cause autosomal dominant parkinsonism with pleomorphic pathology.
Vozdek *et al.*, 2023 [45]	PARC2/ PRKN	Parkin is a 465-amino acid cytosolic E3 ubiquitin ligase involved in proteasome-mediated protein degradation. It damages misfolded and overproduced proteins, as well as ubiquitin. mitophagy regulator.	Absence of Lewy bodies (LBs), apoptosis of dopaminergic neurons in the substantia nigra (SN) and neurofibrillaries
Zhu *et al.*, 2017 [46]	DJ-1 PARC7	Many tissues and organs, including the brain, contain the DJ-1 protein. This protein acts as a chaperone molecule, preventing cells from oxidative stress. DJ-1 assists in the folding of damaged proteins and the assembly of specific proteins into the correct three-dimensional shape. Required for mitochondrial morphology and function	Lewy body disease (LB)
Rauschendorf et al., 2017 [20]	PTEN (ROSE1 PARK6)	Role of PTEN in DNA repair in Parkinson's disease	Since PTEN expression seems to be one of the dominant determinants of neuronal cell death.
Goker-Alpan *et al.*, 2004 [47]	ACS	Glucocerebrosidase	GBA encodes a lysosomal protein that degrades glucocebrosis
Li *et al.*, 2019 [48]	ACSL4	Preferably converts arachidonic acid into acyl-CoA fatty esters, thus playing a key role in ferroptosis	Loss-of-function mutations in ACSL4 deplete lipid peroxidation substrates and increase resistance to ferroptosis
Liu *et al.*, 2022 [49]	LOX	Involved in polyunsaturated fatty acid peroxidation in ferroptosis Tumor suppressor gene	LOX inhibition significantly suppresses cPLA2-dependent microglial induction of reactive oxygen species (ROS) and nitric oxide (NO)
Fol *et al.*, 2016 [50]	APPLICATION	Regulates synapse formation, neuronal plasticity and iron export	APPs*α*, TREM2 and IDE, resulting in decreased levels of Aβ
Dar *et al.*, 2023 [51]	GPX4	Reduces membrane phospholipid hydroperoxides to suppress ferroptose	lipid hydroperoxides accumulating in GPX4-deficient conditions
Islam MT, 2017 [52]	ESG	Glutathione biosynthesis	Loss of function of the glutathione synthetase (GSS) gene leads to 5-oxoprolinuria (pyroglutamic acrithemia)
Ferrari *et al.*, 2023 [53]	CARTE	Microtubule-associated protein; regulates APP trafficking in neurons	The MAPT gene mutation expressed increased levels of 4R-TAU isoforms

PD, Parkinson's disease; SNCA, Synuclein alpha; SN, substantia nigra; LB, Lewy bodies; LC, locus coeruleus; LRRK2, leucine-rich repeat kinase 2; PINK1, PTEN-inducing kinase 1; MAPT, microtubule-associated tau protein; GSS, Glutathione synthetase; GPX4, Glutathione peroxidase 4; APP, amyloid precursor protein; LOX, Lysyl Oxidase; ACSL4, member 4 of the long-chain acyl-CoA synthetase family; GBA, glucosylceramidase beta; PTEN, phosphatase and tensin homologue; DJ-1, Daisuke Junko-1; PRKN, Parkin RBR E3 Ubiquitin Protein Ligase; PARK8, Parkinson's disease-8.

gene into the DNA sequences of cells to treat a disease. The aim of gene therapy is to correct by replacing or complementing a defective mutant allele with a functional one, or to overexpress a protein whose activity would have a therapeutic impact. The modification aims to trigger several types of gene expression. Depending on the case, it may involve inhibiting a defective gene in the patient, compensating for its absence, or even directly repairing a gene through a genome-editing process to make it functional again.

3.3.1. Use of the CRISPR Cas9 System in Gene Therapy for Parkinson's Disease

The CRISPR Cas9 system is a powerful technology for rapidly modifying the target genomic sequence. More and more scientists are using this technique to conduct research into neurodegenerative diseases such as Parkinson's. Candidate genes targeted for CRISPR Cas9 gene therapy in PD include SNCA, LRRK2, VPS35, HTRA2, EIF4G1, GBA, PARK2, PINK1, PARK7, ATP13A2, PLA2G6 and FBXO7. Some genes are thought to increase the risk of developing PD, while others reduce the risk. SNCA has several mutations, but A53T is particularly notable for its association with Parkinson's disease. Indeed, Yoon *et al.*, (2022) [23] had used the CRISPR-Cas9 system to delete the A53T-SNCA gene to ameliorate Parkinson's disease-related conditions such as beta-synuclein overproduction, reactive microgliosis, dopaminergic neurodegeneration and motor symptoms associated with Parkinson's disease. In contrast, Chen *et al.*, (2020) [24] have investigated the mechanism by which SNCA operates in the nucleus using neurons derived from pluripotent cells from Parkinson's disease patients possessing A53T and autosomal dominant SNCA triplication mutations, as well as their CRISPR-edited corrected counterparts. The study demonstrated that the absence of SNCA leads to resistance to Lewy pathology, indicating the possibility of using CRISPR/Cas9n-mediated gene editing as a potential treatment for Parkinson's disease. In another study, Zhou *et al.*, 2015 [25] were able to successfully edit the PARK2 and PINK1 genes that induce PD using the CRISPR-Cas9 system. In addition, fascinating research has been carried out by Ahfeldt *et al.*, 2020 [26] on nigral dopaminergic neurons (DN) using the CRISPR/Cas system to suppress the PARKIN (PRKN), DJ-1 (PARK7) and ATP13A2 (PARK9) genes that cause PD [27]. The GBA gene is responsible for encoding the lysosomal hydrolase glucocesocrebroosis, an enzyme that hydrolyzes the sphingolipid waste glucosylceramide to ceramide and is a documented risk factor for PD [28]. Hanss *et al.* 2020 [29] were able, using the CRISPR Cas 9 system, to edit the GBA gene that induces PD (**Table 2**). Thus, iPSCs, CRISPR/Cas9, CRISPR-iPSC and MicroRNA-based approaches are accelerating research into neurodegenerative diseases and bringing us closer to gene therapy for neurodegenerative diseases such as Alzheimer's, Huntington's, amyotrophic lateral sclerosis and Parkinson's disease.

3.3.2. MicroRNA Gene Therapy for Parkinson's Disease

Encoded by the genome, then transcribed into a precursor in the form of a

Table 2. Overview of studies on PD models using CRISPR Cas9, associated editing strategies and ethics.

References	PD model	Target gene	Mains concequences	Risks of using CRISPR/Cas9 in gene therapy and Ethics
Hsieh *et al.* 2016 [54]	iPSC of a parkinsonian patient	LRRK2	Delayed mitochondrial autophagy and impaired cellular respiration and metabolism.	Limitations of CRISPR-Cas9 systems include the efficacy of Cas9 delivery to cells or tissues, off-target effects and ethical concerns regarding the use of CRISPR technology in humans. Adenoviral vectors (AdVs) are frequently used in clinical trials to deliver genes. AdVs can target both non-dividing and dividing cells. Genome-editing techniques used in gene therapy can sometimes be imprecise and uncontrollable, leading to unexpected and unpredictable effects. These genetic errors, including "on-target" and "off-target" effects, can lead to unexpected results in genome editing. CRISPR can also induce double-strand breaks (DSBs) in target DNA and, through genetic breaks, can trigger apoptosis rather than the intended genetic modification. In the application of gene therapy using viral vectors, we must apply not only the ethical precautionary principle but also the fundamental bioethical principles of beneficence and non-maleficence.
Imaizumi *et al.*, 2012 [55]	iPSC of a parkinsonian patient	PARC2	Increased oxidative stress accompanied by activation of the Nrf2 pathway; Abnormal mitochondrial morphology and impaired mitochondrial turnover.	
Ishizu *et al.* 2016 [56]	Mice	VPS35	Survival disadvantage and DA release is significantly reduced in the caudate putamen.	
Xu *et al.*, 2023 [57]	Mice	CDK5	Deficits in locomotor activity and disturbances in activity/rest behaviour, down-regulation of dendrite length and reduction in the number of functional synapses in the brain.	
Zhu *et al.* 2018 [58]	Pig	SNCA	No obvious neuronal loss, normal behavior.	
Yao *et al.*, 2014 [59]	Pig	PARC7	DJ-1 protein was repressed in all tissues detected and all pigs died due to a cloning defect.	
Wang *et al.* 2016 [60]	Pig	Parkin/DJ-1/ PINK1	No obvious neuronal loss, normal behavior.	
Yang *et al.* 2019 [61]	Monkey	ROSE1	Some died after birth and the surviving monkeys showed severe degeneration and death of neural brain cells.	
Li *et al.* 2021 [62]	Monkey	ROSE1 et DJ-1	Severe loss of dopaminergic neurons and accumulation of synaptic nucleoprotein pathology in substantia nigra	

stem-loop, microRNAs are made up of around twenty nucleotides and form one of the major pathways regulating gene expression. Matured in this way, microRNAs can regulate gene expression, by pairing with messenger RNAs carrying a homologous sequence to degrade them or inhibit their translation. MicroRNAs can also deactivate a gene by methylating it.

1) ***The role of microRNAs in the pathogenesis of Parkinson's disease***

Today, several studies have demonstrated that microRNAs (miRNAs) are capable of negatively regulating gene expression. They may also play a key role in the pathogenesis of PD [30]. Indeed, it has been shown that various miRNAs directly down-regulate the SNCA gene, their aberrant expression causing α-Syn deposition and neuronal cell death [31]. Furthermore, it has been demonstrated in in vitro models of PD that accumulated expression of miR-7 and miR-153 can

exert a neuroprotective action on dopaminergic neurons [31].

2) ***The exosome/miRNA system: a potential gene therapy for Parkinson's disease***

With the progress of genomic sciences, the exosome/miRNA system could become a potential target gene therapy for Parkinson's disease.

According to Paccosi and Proietti-De-Santis, (2023) [17], The main obstacle to the development of new drug treatments for Parkinson's disease and neurodegenerative disorders in general is getting therapeutic molecules to cross the blood-brain barrier (BBB). However, it should be noted that exosomes, which are secreted vesicles produced in the endosomal compartment, are capable of shuttling between the cells of the endosomal compartment [32]. Suggesting that they may be potential vehicles for delivering drugs to the brain [33]. According to Paccosi and Proietti-De-Santis (2023) [17], modified exosomes, separated from different cell types, are able to target specific brain regions and determine neuron types, opening up a promising scenario for the treatment of Parkinson's disease and other neurodegenerative pathologies. Indeed, it has been demonstrated that exosomes, as paracrine factors, contain a large number of miRNAs, DNA fragments, proteins and other bioactive molecules, and are capable of shuttling between cells and modifying the physiological functions of these same cells [34]. In this context, many stem cell types, such as bone marrow mesenchymal stem cells (BM-MSCs), human embryonic mesenchymal stem cells (hES-MSCs) and induced pluripotent stem cell-derived neural progenitor cells (iPSC-NPCs), have been reported to protect neurons from ischemic stroke-induced death [35]. Clinical trials using MSCs for the treatment of PD are currently underway worldwide [36].

A promising strategic prospect for the treatment of Parkinson's disease has just opened up to researchers: the potential exploitation of modified exosomes, which can be loaded with bioactive molecules such as therapeutic compounds and RNAs, enabling them to be delivered to the appropriate location in the brain, overcoming cerebral barriers [17]. As proof, BM-MSC-derived exosomes contain certain miRNAs, including miR-146a, miR-133b and miR-21, and are capable of enhancing neuronal plasticity and promoting cell survival [37]. However, despite the strong potential demonstrated by exosomes as transport vehicles for miRNAs capable of modifying gene expression to alter the prognosis or progression of PD, efforts remain to be made. **Table 3** presents the target genes, miRNA regulatory mechanisms and ethics of gene therapy.

3.4. Assessing the Risks and Benefits of Gene Therapy for Parkinson's Disease Based on Bioethical Principles

Gene therapy trials for Parkinson's disease can be weighed up by assessing the risks, limitations and benefits of therapeutic protocols. The symptoms of PD are listed above, and include bradykinesias, slow involuntary movements, tremors, gait disorders, imbalance and rigidity, cognitive impairment, depression and

Table 3. Target genes, miRNA regulatory mechanisms and gene therapy ethics.

Authors	MiRNAs negatively regulated in PD	Target genes	Molecular mechanisms	Ethics and the use of miRNAs in gene therapy
McMillan *et al.*, 2017 [63]	miR-7	*SNCA*	MiR-7 is responsible for down-regulating SNCA gene expression, its depletion being associated with α-Syn accumulation and neuronal loss.	The current technology for obtaining exosomes needs to be improved in order to obtain purer exosomes: technologies for their purification and enrichment are still fairly rudimentary. Another reasonable concern stems from quality control of exosome purity, which remains an open challenge: there is no candidate protein that is a unique and specific marker for exosomes. Until recently, the most common method for loading miRNAs or siRNAs into exosomes was electroporation. Although this strategy has proved effective for loading siRNAs into purified exosomes, unfortunately, transfection of exosomes directly with nucleic acid using this system is not entirely efficient, as it requires both separation and purification of exosomes before and after transfection, thus drastically reducing the quantity of exosomes. Another limitation of exosomes is that they are usually obtained in small yields. Exosomes cannot be stored for long periods. Consequently, there is also a need to improve exosome preservation technology to protect their biological activities and make them suitable for clinical application. Thus, the use of microRNAs in gene therapy can damage the human organism. Their use in gene therapy therefore requires caution and scrupulous respect for the ethical principles of precaution and the fundamental bioethical principles of beneficence and non-maleficence: "Primum, non nocere"!
Zhang *et al.*, 2018 [37]	miR-21	*PTEN*	MiR-21 enhances neuronal plasticity and cell survival.	
Asadi *et al.*, 2023 [64]	miR-873	*ABCA*1	MiR-873 is able to reduce PD symptoms by up-regulating ABCA1 and A20. Inhibition of miR-873 may play a dual protective role in PD by inducing intracellular cholesterol homeostasis and ameliorating neuroinflammation.	
Straniero et al., 2017 [65]	miR-22-3p	*ACS*	MiR-22-3p controls GBA.	
Doxakis et al., 2010 [66]	miR-153	*SNCA*	MiR-153 is responsible for down-regulation of SNCA gene expression, its depletion inducing α-Syn accumulation and neuronal loss.	
Miñones-Moyano et al., 2011 [67]	miR-34b/c	*SNCA, Parkin* et *DU*-1	MiR-34b/c is responsible for the down-regulation of SNCA gene expression, its depletion leading both to the deposition of α-Syn in PD brain tissue and to the down-regulation of Parkin and DJ-1 gene expression.	
Kim *et al.*, 2007 [68]	miR-133b	*Pitx*3	MiR-133b is specifically expressed in the midbrain, where it regulates both the maturation and function of midbrain DA neurons, its depletion being associated with a massive loss of DA neurons.	
Soreq et al., 2011 [69]	miR-124	*Calpain* 1, *Bim, STAT*3, *Annexine A*5, *MEKK*3	MiR-124 regulates synapse morphology, neurotransmission, inflammation, autophagy and mitochondrial function, its depletion being implicated in major pathophysiological mechanisms.	
Chen *et al.*, 2017 [70]	miR-4639-5p	*DJ*-1	MiR-4639-5p negatively regulates DJ-1 post-transcription levels, its up-regulation being responsible for massive induction of oxidative stress and, consequently, neuronal death.	
Jiang *et al.*, 2019 [71]	miR-137	*OXR*1	MiR-137 is involved in the induction of oxidative stress in neurons, its up-regulation being implicated in a massive induction of oxidative stress and neuronal death	

dementia. At present, there is no effective medication for PD. Hope for a cure is likely to come from gene therapy. But as with any modern technology, there is no such thing as zero risk in applied gene therapy. There are advantages and potential risks and disadvantages. Should we let these patients die a slow death without trying out these revolutionary new therapeutic techniques? Or should therapeutic trials be carried out, notwithstanding the potential risks? But is everything that is technically feasible ethically acceptable? Hence the application of the precautionary principle, demands that researchers and healthcare professionals do not set foot where they are not sure. Indeed, the bioethical principle of non-maleficence recommends: "primum non nocere". If you can't fix or improve a pernicious situation, don't make it worse. Certainly, gene therapy that corrects mutated genes or employs CRISPR Cas9 and microRNA technologies has advantages and potential therapeutic results, as it may slow the development of the disease, or even eradicate it. As in all pharmaco-clinical trials, the application of gene therapy requires respect for fundamental bioethical principles such as:

3.4.1. The Principle of Autonomy

For healthcare professionals, the principle of autonomy means respecting patients' freedom of choice and decisions: it means respecting their self-determination [38]. This principle includes the concepts of free and informed consent, confidentiality, medical secrecy and respect for human dignity [39]. One of the cornerstones of the principle of autonomy is genetic counselling, which is the process by which patients or their relatives are taken into therapeutic care. It is the first step in enabling patients to assume their autonomy by giving their free and informed consent to a therapeutic trial. Autonomy is an essential ethical principle in medical practice, in this case in the case of Parkinson's disease. However, in the case of a neurodegenerative disease, where cognitive abilities may be impaired, obtaining free and informed consent can be more complex. It is important to consider the ethical standards of informed consent for adults and minors with cognitive impairment. Prior to PD Gene Therapy, patients must be informed of the risks and benefits of the therapy to be applied, as well as how the results will be used and shared to enable patients to freely give informed consent before gene therapy is initiated [8]. All the more so as genetic information is highly sensitive and must be handled with caution, care and discretion. Healthcare professionals must protect the genetic information of PD patients with the utmost confidentiality, and disclose it only with the explicit consent of adult patients. Their free and informed consent is also required to inform their loved ones of the situation. Digitized and stored genetic information is subject to medical confidentiality, and must be treated with care and discretion to protect the privacy and dignity of the person affected by PD.

3.4.2. The Principle of Beneficence

The principle of beneficence is an ethical concept that emphasizes the importance

of acting in the best interests of patients and providing them with the best medical care whenever possible. Beneficence, in the case of PD treatment, would be to provide patients with appropriate and adequate therapy to help slow disease progression and improve their quality of life [40]. Once the diagnosis has been established, treatment options can be considered, such as specific drugs to help control the symptoms of the disease. From this perspective, if no conventional medication can provide relief for the PD patient, then gene therapy may be an option if, and only if, this new therapy has followed clinical trial protocols and does not worsen the health of the person affected by PD. However, this new therapy must not only respect the confidentiality and dignity of the patient, but also take into account the values, wishes and interests of the patient and his or her family.

While the application of gene therapy for PD has its advantages, it also has its limitations, risks and drawbacks. Genome-editing techniques are sometimes imprecise, uncontrollable and could cause unexpected and unpredictable effects. During genome editing, genetic errors, such as "on-target" and "off-target" effects, could be committed, leading to unexpected manipulation results. Genome editing can inadvertently cause extensive deletions and complex rearrangements of DNA segments in the organism whose genome has been edited. Unwanted DNA segments can be unintentionally integrated into the host organism's genetic make-up during the genome-editing process. For example, foreign DNA has unexpectedly found its way into the ectopically edited genome. While microRNAs can regulate gene expression, by pairing with messenger RNAs carrying a homologous sequence to degrade them or inhibit their translation. They can also deactivate a gene by methylating it. Thus, the use of microRNAs in gene therapy can damage the human organism. Their use in gene therapy therefore requires caution and scrupulous respect for the ethical principles of precaution and the fundamental bioethical principles of beneficence and non-maleficence.

3.4.3. Le Principe de Non-Malfaisance

The principle of non-maleficence, known as "primum non nocere", is a fundamental ethical principle in therapeutic medicine. It emphasizes the importance of avoiding harm, injury or endangerment to a patient's life, and of ensuring that the patient's best interests are taken into account during medical treatment [40]. With this in mind, healthcare professionals must avoid causing harm to patients, including by avoiding providing them with inaccurate or misleading information that could mislead them or cause them unnecessary stress [41]. In the case of neurodegenerative diseases such as PD, it is important to carefully weigh the potential benefits of therapies against the possible disadvantages [41]. Healthcare professionals must be prepared to provide clear information and support family members throughout the decision-making process. Patient-specific treatment protocols should only be shared with those authorized by the patient, such as family members. In accordance with the principle of non-maleficence, every effort must be made to avoid physical, psychological and social harm to the patient's health in the department where the PD patient is treated for gene therapy?

3.4.4. The Principle of Justice

The notion of universal justice in the field of therapeutics refers to the idea that all individuals should have access to quality medical services, regardless of their social or economic background. This means that all patients should have equal access to diagnostic testing, treatment and care, without discrimination or injustice based on factors such as race, age, gender, or socio-economic status. In the case of PD, special therapy and care such as proven effective gene therapies must be accessible to all people with PD, regardless of ability to pay or social status. Healthcare professionals must work to reduce healthcare inequalities and promote fairness, impartiality and equity [42].

4. Conclusion

Our literature search has helped to highlight advances in the fields of genetics and epigenetics, which have led to the discovery of numerous genetic mutations and polymorphisms responsible for PD. However, despite advances in genetic screening and research, there is still no certified, effective treatment for patients with dementia. The subject of gene therapy for PD raises important questions concerning bioethical principles such as autonomy, beneficence, non-maleficence and justice. Notwithstanding all these risks of applied gene therapy in the face of its potential therapies, it's important to know how to take risks. Because nothing ventured, nothing gained. It is therefore essential to pursue research and ethical discussions to advance treatment options and meet the challenges of brain manipulation and its impact on human identity. The golden rule of medicine remains the same: "*Primum non nocere*".

Ethics Approval and Consent to Participate

The HOSCO/CERBA Institutional Ethics Committee approved this study in Deliberation No. 2023-02-09 of 15 February 2023. Study was conducted according to the declaration of Helsinki.

Consent for Publication

All authors have read and approved the manuscript for publication.

Availability of Data and Materials

Not applicable.

Authors' Contributions

TMZ, JS, AAZ, AKO, JS, designed the study.

JS, TMZ, MNKO, FWD, CWMN, RK, DO, wrote the manuscript.

JS, TMZ, PO, FWD, DO, collected the data.

JS, TMZ, computerized the data.

JS, TMZ, MNKO, FWD, CWMN, RK, DO, RO, DOY, revised and edited the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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