Parkinson's Disease

Pauwels, Ernest K.J.; Boer, Gerard J.

2023

DOI (link to publisher)
10.1159/000531422

document version
Publisher's PDF, also known as Version of record

Medical Principles and Practice

Review

Med Princ Pract 2023;32:155–165
DOI: 10.1159/000531422

Received: March 13, 2023
Accepted: June 1, 2023
Published online: June 7, 2023

Parkinson's Disease: A Tale of Many Players

Ernest K. J. Pauwels[a] Gerard J. Boer[b]

[a]Leiden University and Leiden University Medical Center, Leiden, The Netherlands; [b]Netherlands Institute for Brain Research, Royal Academy of Arts and Sciences, Amsterdam, The Netherlands

Highlights of the Study

- The number of patients with Parkinson's disease is increasing worldwide.
- Current treatments do not stop disease progress.
- Preclinical studies pave the way to new treatments.
- Clinical treatments under development include synolytics, gene editing, cell transplantation, targeted physiotherapy.

Keywords
Parkinson's disease · Alpha-synuclein · Synolytics · Cell-based therapy · Targeted physiotherapy

Abstract
In 2020, more than 9 million patients suffering from Parkinson's disease (PD) were reported worldwide, and studies predict that the burden of this disease will grow substantially in industrial countries. In the last decade, there has been a better understanding of this neurodegenerative disorder, clinically characterized by motor disturbances, impaired balance, coordination, memory difficulties, and behavioral changes. Various preclinical investigations and studies on human postmortem brains suggest that local oxidative stress and inflammation promote misfolding and aggregation of alpha-synuclein within Lewy bodies and cause nerve cell damage. Parallel to these investigations, the familial contribution to the disease became evident from studies on genome-wide association in which specific genetic defects were linked to neuritic alpha-synuclein pathology. As for treatment, currently available pharmacological and surgical interventions may improve the quality of life but do not stop the progress of neurodegeneration. However, numerous preclinical studies have provided insights into the pathogenesis of PD. Their results provide a solid base for clinical trials and further developments. In this review, we discuss the pathogenesis, the prospects, and challenges of synolytic therapy, CRISPR, gene editing, and gene- and cell-based therapy. We also throw light on the recent observation that targeted physiotherapy may help improve the gait and other motor impairments.

karger@karger.com
www.karger.com/mpp

Correspondence to:
Ernest K. J. Pauwels, ernestpauwels@gmail.com

Introduction

The longer we live, the greater the risk of age-related diseases, including neurological conditions like Parkinson's disease (PD) [1]. Indeed, worldwide in 2020, more than 9 million patients suffering from PD were reported. Various studies predict that the burden of this disease will grow substantially in industrialized countries. For instance, a Norwegian study found that PD prevalence increases with age, peaking at 85 years. Moreover, the incidence ratio changed from 1.4 in people at 60 years to 2.0 in those older than 90 years [2].

In the last few decades, there has been a better understanding of the neuropathology and characteristics of PD that leads to this disabling disease with symptoms such as motor disturbances, impaired balance and coordination, memory difficulties, impaired speech, and lack of impulse control. A hallmark of the disease is the major loss of dopaminergic (DA) neurons in the substantia nigra (SN), leading to dysfunction of the nigra-striatal motor pathway [3]. The main pathological characteristic of PD is the presence of Lewy bodies which are cytoplasmic inclusions confined to nerve cells, also seen in other neurodegenerative diseases. Autopsy results of PD patients revealed that Lewy bodies are found mostly in areas of neuronal loss. Spillantini et al. [4] were among the first to provide evidence that a small protein called alpha-synuclein is present in these structures. This finding has placed this compound at the center of ongoing research [5].

Many preclinical studies made it clear that an inflammatory cerebral environment promotes misfolding of alpha-synuclein and aggregation in Lewy bodies. The senescence-associated secretory phenotype (SASP) plays a major role due to the secretion of inflammatory cytokines, chemokines, growth factors, and proteases in the absence of any obvious pathogen. Disturbances in synaptic transmission led to multiple dysfunctions of the CNS as is the case in PD. An excellent and well-illustrated article by Sikora et al. [6] discusses the role of cellular senescence in brain aging. Importantly, mitochondrial age-related dysfunction is an epiphenomenon of senescence and may even function as a driver of the phenotype. Mitochondria are one of the key modulators in the development of the senescence phenotype, particularly the SASP, entailing the pro-inflammatory environment [7, 8]. Leakage through the membrane releases mitochondrial enzymes and reactive oxygen species. This aggravates cellular senescence by enhancing DNA damage and its restoring pathways [8, 9]. Parallel to the abovementioned research, the molecular contribution to PD genetics will become evident from worldwide association studies.

This review provides an overview of critical findings from recent studies. We systematically examined the existing literature, and we outlined the role of major players as synuclein, microglia, astrocytes, and senescence in PD-associated neurodegeneration. Studies to identify genes associated with susceptibility to PD are highlighted, and we discuss how this knowledge will contribute to the development of a risk prediction model. We conclude with a summary and discussion on the status of therapies in view of recent (pre-) clinical developments. For this, we have chosen to describe in a transparent way the major features of the most important biochemical players and therapeutic advances in PD, rather than discuss in detail the different research strands.

Data Collection

A survey of peer-reviewed literature in the English language, retrieved from the medical database of PubMed and Cochrane Library was conducted for all years of publication, using the keywords PD, pathology, current treatment, treatment developments, and prognosis. Both original articles and review papers on preclinical investigations and human studies were included. Editorial pieces and commentaries were excluded. The two authors of this article screened the articles that were identified based on titles and abstracts. Using this method, the full text of relevant articles was retrieved from the databases after which an independent selection of relevant texts for final inclusion in this review took place. In case of differences between the two authors, article inclusion was resolved by discussions and consensus. Finally, critically appraised papers were used for this review.

Alpha-Synuclein

Alpha-synuclein is a presynaptic neuronal protein that controls neurotransmitter release and is an important regulator of dopamine homeostasis [10]. This small protein is encoded by the SNCA gene and consists of 140 amino acids with an unstructured C-terminal tail at 96–140 [11]. In the brain of PD patients, it is predominantly expressed in the neocortex, hippocampus, thalamus, and cerebellum [12]. After the discovery of cytoplasmic synuclein, its actual function has long been elusive. Schaser et al. [13] and Provasek et al. [14] revealed at least part of its intracellular roles, using an in vivo imaging approach. It was found that Lewy body inclusion containing neurons showed an increased level of nuclear double-strand breaks and that alpha-synuclein is rapidly recruited to these sites to induce DNA repair. Similarly, in alpha-

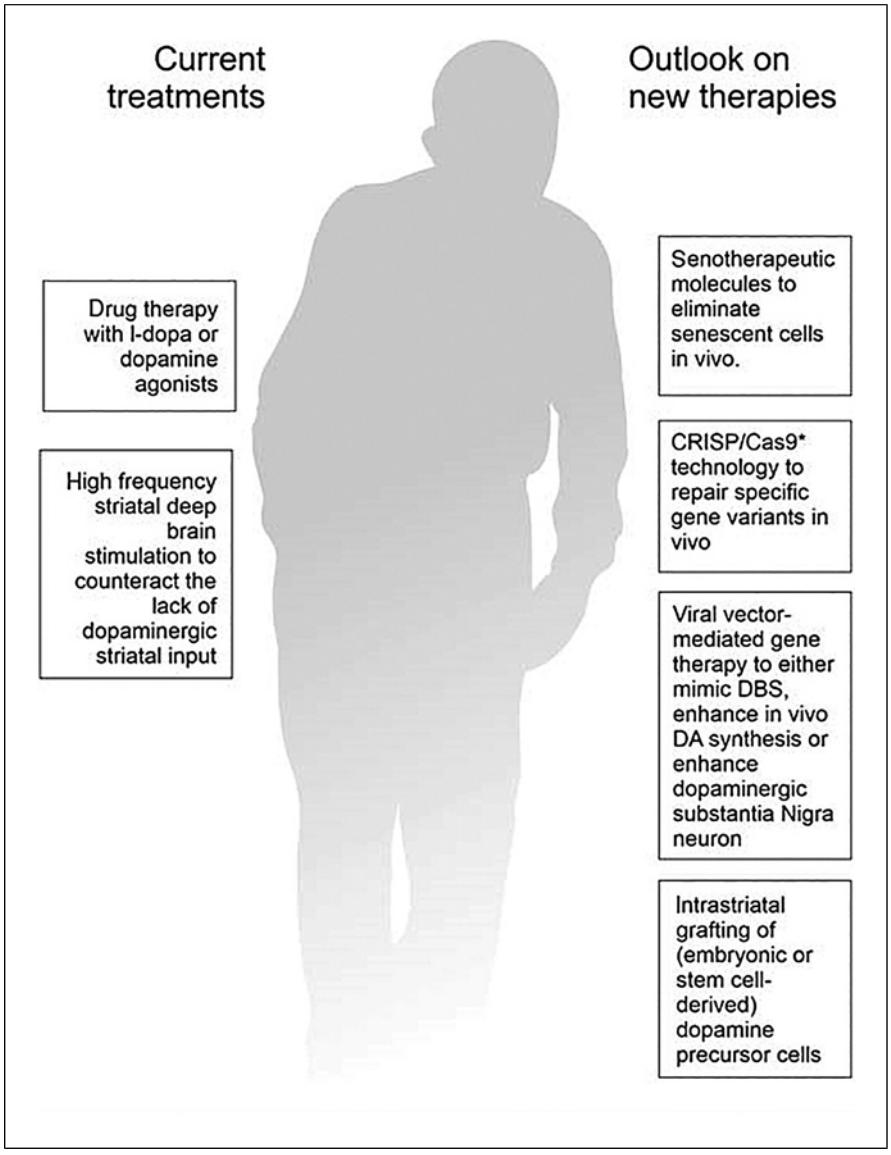

Fig. 1. Overview of current therapies (left) and work in progress (right) to possible future therapy for Parkinson's disease. DBS, deep brain stimulation; DA, dopaminergic neuron.

synuclein knockout mice, it was demonstrated that increased neuronal double-strand breaks can be rescued by transgenic reintroduction of human alpha-synuclein. It is likely that an imbalance between double-strand breaks and dysfunctional alpha-synuclein, caused by protein misfolding and formation of cytoplasmic aggregates in Lewy bodies mediated by C-terminal truncation, can lead to loss of neuronal function (Fig. 1). This is not inconsistent with the observation that in sporadic PD patients' excessive presence of alpha-synuclein has been demonstrated in presynaptic terminals as well as in dendritic spines [15, 16].

It is presently not fully known how cytoplasmic aggregation of alpha-synuclein into Lewy body pathology causes neuronal death [17, 18]. However, the biochemical mechanistic process that leads to Lewy body formation, neuronal dysfunction, and degeneration has been made less elusive by Mahud-Melliera et al. [19]. In meaningful experiments, these researchers have demonstrated that Lewy body formation involves a complex process in which *exogenously* preformed fibrils of alpha-synuclein can act as seeds to initiate the misfolding and aggregation of *endogenous* alpha-synuclein. This has also been suggested previously by Volpicelli-Daley et al. [20]. In short, using electron microscopy, it was observed that these initial aggregates, heterogeneous in size and shape, were observed in neuritic inclusions, but do not share the structural and morphological features of bona fide

Lewy bodies. Hypothetically, the subsequent sequestration of lipids, organelles, membrane structures, and mitochondrial proteins lead to structures that morphologically resemble the bona fide human Lewy bodies. These experiments have not clarified the intermediate biochemical steps, but the results demonstrate a likely part of the mechanism that leads to neurodegeneration.

Microglial Cells

Microglia are immunocompetent macrophages surveilling the brain and are responsible for homeostasis. They scavenge dead cells and provide structural support. The mechanism of autophagy has recently been described by Fellner et al. [21]. This chaperone-mediated pathway via lysosomes is age-related. Reduced autophagy results in diminished homeostasis and propagation of neuroinflammation through senescence of glial cells. Moreover, through surface receptors, microglia can become activated by exogenous and endogenous triggers, such as injury and migration [22]. In this activated stage, they release inflammatory cytokines and reactive oxygen species leading to DA neuron death due to aggregated alpha-synuclein mediated by nuclear factor B [23]. Interestingly, activated microglia can also create a neuroprotective anti-inflammatory ambience [24].

Astrocytes

Astrocytes, like microglia, have a prominent role in the homeostasis of the cerebral environment. Healthy cells actively communicate with neurons, microglia, and other astrocytes [25]. With age, astrocytes may turn into promotors of neuroinflammation, releasing chemokines, cytokines, growth factors, prostaglandins, and nitric oxide, whereas phagocytosis, neuronal survival support, and synaptogenesis are no longer functional. This transformation into "reactive astrocytes" (often called A1 phenotypes) is present in human neurodegenerative diseases and is neurotoxic to a high degree [26]. In contrast, the so-called A2 phenotype astrocytes have a protective repair function after ischemia [27].

In relation to PD, Joe et al. [28] have summarized important properties linked to the surveying function of astrocytes throughout the brain. To name a few, these cells inhibit inflammatory microglial activation, regulate the function of the blood-brain barrier, and regulate the phagocytosis of non-functioning synapses. It is evident that astrocytes are critical for normal brain function, while pathological conditions related to aging negatively affect the inflammatory response and the surveillance function of these cells. Thus, dysfunction of the astrocytes plays an important role in the onset of PD [29].

Senescence

A prominent contributor to aging is cellular senescence that imposes irreversible and permanent cell cycle arrest. With aging, senescent cells accumulate in various tissues and at pathological sites [30, 31]. The accumulation of senescent cells in organs including the brain, results in functional impairment due to changes in dendritic spines, essential for synaptic function. In the brain, this is linked to cognitive deterioration. Senescent cells entail apoptotic resistance, and 30–70% of the cells can turn into a pro-inflammatory SASP. The SASP contributes to many chronic diseases, including PD [32]. Recently, Russo and Riessland [33] hypothesized that secretion of pro-inflammatory factors by senescent cells in the midbrain triggers neuroinflammation, resulting in immune cell-mediated killing of midbrain DA neurons in PD. Although the SASP of senescent cells creates a tissue-destructive pro-inflammatory environment, the cell itself is not affected. Zhu et al. [34] made clear that senescent cells can upregulate a pro-survival pathway that protect them from the pro-apoptotic environment they create around themselves. Indeed, the composition of SASP depends on the cell type from which the senescence originates. Furthermore, it appears that other factors can determine the modifiability of SASP. These include the presence of drugs like metformin [35] and corticosteroids [36], which exert a suppressive effect on senescence-associated inflammation and apoptosis.

Genetic Etiology

The completion of the Human Genome Project in 2003 has been instrumental in making gene maps and sequences available to study abnormalities from biochemical, physiological, and functional standpoints. The identification of causal genetic factors in PD relies heavily on the results of this project and classifies abnormalities into familial and sporadic groups [37, 38]. Against this background, genome-wide association studies (GWAS) are revealing risks and variants of PD. Using microarray technology, it has been discovered that the alpha-synuclein (SNCA) and leucine-rich repeat kinase 2 (LRRK2) genes are important players in inherited and sporadic PD for all races. A study by Nuytemans et al. [39] described five major genes and proteins related to PD: the autosomal dominant genes SNCA and LRRK2 and the autosomal recessive genes PINK1 (beta-TEN-induced putative kinase 1), PARK2 (parkin), and DJ-1 (also called PARK7). The mapping of these genes can be found on Entrez Gene (http:/www.ncbi.nlm.nih.gov/gene). To date, by analyzing the data of thousands of patients, at least 24 PD-associated genes have been

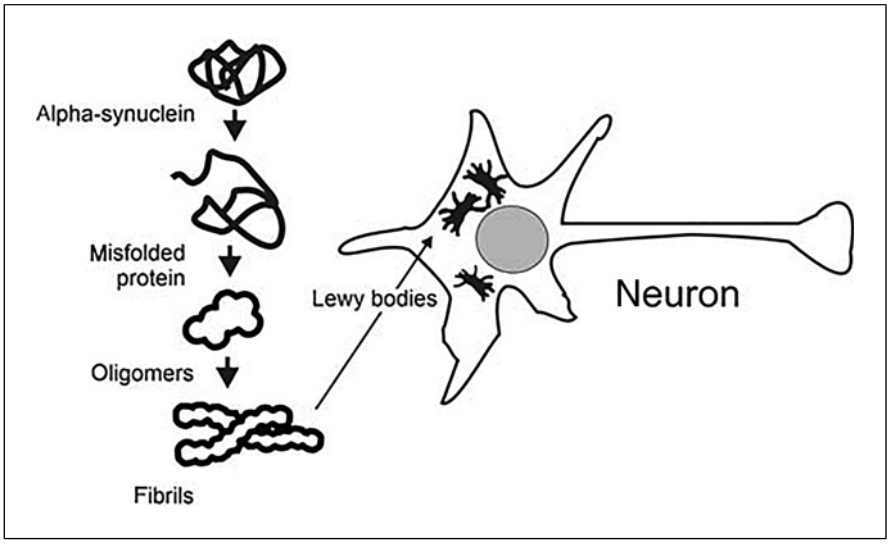

Fig. 2. Schematic outline showing the pathway in which alpha-synuclein turns into misfolded protein and oligomers. This results in the formation of cytoplasmic Lewy body, ultimately leading to loss of neuronal function.

detected. A meta-analysis of 17 genome-wide association studies available from European ancestry data studied data from 7.8 million single-nucleotide polymorphism and 1.4 million controls [40]; 90 independent risk signals across 78 genomic regions, including 38 novel risk signals in 37 loci, were discovered. However, only 16–36% of these 90 variants explained heritable risk of PD. Furthermore, Mendelian randomization between cognitive performance and risk for PD showed a very robust association ($p = 8 \times 10^{-7}$). Additional information on the genetic architecture of PD has been presented by Yao et al. [41]. These investigators integrated GWAS results with large-scale expression loci in 13 brain samples to identify causal PD genes in a Chinese population; their approach discovered four new, previously non-prioritized genes at known PD loci. Of note is the fact that the genetic background significantly affects the clinical disease course and severity. This has been evidenced by longitudinal cohort data analysis on the relationship between genetic risk factors and disease characteristics [42].

Mahmut-Melliera and colleagues [19] investigated transcriptomic changes occurring over 3 weeks; they found that the expression level of 217 genes related to PD changed dramatically between two and 3 weeks. Also, over this period, a dramatic reduction of pre- and postsynaptic markers was noticed. This was also the time span in which the inclusion processes, the Lewy body maturation, and neuronal cell death occurred. Thus, it appeared that synaptic dysfunction is primarily linked to Lewy body-like inclusions. It has been rightly suggested that this neuronal model (Fig. 1) mirrors the key biochemical, structural, and organizational features of Lewy body pathologies in PD brains [43–45]. In the following two sections of this review, we will discuss the current therapies applied for PD and provide an outlook on therapies under development (Fig. 2).

Current Therapies

Drug Therapy

The current therapy with drugs aims to manage the symptoms of PD patients. The ability of levodopa to increase the dopamine level in the brain has been well established. Its safety and tolerability profiles of this DA precursor have made it widely applicable. However, long-term use of this drug finally can lead to levodopa-induced and badly controllable dyskinesia, showing the limitations for treatment in the final stages of PD.

Known DA agonists are applied to enhance DA activity to control the symptoms, usually in combination with a MAO-B (monoamine oxidase B) inhibitor slowing down the breakdown of striatal dopamine. These include drugs such as bromocriptine, pergolide, pramipexole, cabergoline, and apomorphine. No other drugs have similar or better disease-modifying properties, although slight modifications in the use of the abovementioned drug are presently under investigation. For example, an extended release-combined formulation of pramipexol and rasagiline (MAO-B inhibitor) is being tested in a phase 3 trial in 544 patients and appears to show superiority compared to its individual components, whereas the safety profile is similar to a placebo [46].

Deep Brain Stimulation

A meta-analysis of the deep brain stimulation (DBS) surgery technique to alleviate symptoms in PD patients has been published recently [47]. A recent multicenter

double-blind, randomized, and MASH-controlled study has shown safety and clinical efficacy in 196 patients before randomization and compelling clinical improvement was observed between the active and control group ($p < 0.0001$) [48].

One important question is how long a beneficial effect of this intervention will persist. A study on long-term health outcomes by Bove et al. [49] was based on a complete data set of 51 patients comparing the preoperative stage with data obtained at 1 year and at 17 ± 2 years. This well-documented study (in which a similar control group is lacking for obvious reasons) has demonstrated that over the entire period of study, dyskinesia and time in the off state improved considerably. Moreover, improvements in the quality of life, both emotional and social function, as well as a significant reduction in DA treatment were observed. In contrast, in various other follow-up studies, cognitive scores over the study period less than 15 years significantly worsened, evident as early as at 1 year after surgery. In this respect, the ethical question regarding patients who do not experience improved quality of life is paramount. This situation may come with unexpected stages of PD, like emerging iatrogenic harm for patient and difficulties for family members and caregivers [50].

Outlook on Therapies

Senolytics

The targeting and selective clearing of the harmful spreading senescent cells is an obvious approach to fight age-related brain diseases, including PD and other dementias. Thus, the critical removal of senescent cells is believed to improve physical and cognitive function. Middle-aged mice treated with a cocktail of dasatinib and quercetin, considered a senolytic anti-inflammatory drug was observed to have a delay in old age-related ailments [51]. Conversely, these beneficial effects were not noticed in a control group. Furthermore, this first drug with senolytic properties effectively cleared senescent cells and did not affect the normal cells around them. An extensive review on the historical biomedical development of these senolytic drugs has recently been published by Chaib et al. [51].

This interesting approach was pursued by Kirkland and Tchkonia [52]; they described efforts to target senescent cell anti-apoptotic pathways (SCAPs) by senotherapeutic molecules to eliminate senescent cells in diabetic kidney disease. Since then, a plethora of papers have reported results obtained in preclinical murine studies centered around SCAPs, but the relevance for the disease process of PD is still to be confirmed [51].

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Gene Editing

The therapeutic targeting of the specific genes and mutations, linked to individual PD patients, would open the possibility to treat PD patients in a tailored way. Gene editing using CRISPR/Cas9 technology to "repair" specific gene variants has opened this door. Basically, this technology uses a DNA-cutting enzyme Cas9 and short guide RNA strands that directs the enzyme to the target, in which the genome can be edited, by inserting or deleting DNA sequences. This technique has the potential to cure genetic diseases. One example, regarding the PD etiology, is in which CRISPR/Cas9 genome editing may help protect neurons against oxidative stress induced by defective mitochondria. Under normal circumstances, damaged mitochondria are eliminated in a process in which the two genes PINK1 and parkin play an important role.

PINK1 regulates parkin translocation in defective mitochondria and removes them in a selective autophagic process, known as mitophagy. Disruption of PINK1/parkin signaling in PD allows the accumulation of harmful mitochondria, resulting in deficient synaptic function [53, 54]. A detailed overview of gene-editing technology was recently described by Rachman et al. [55]. To prepare for the step from bench to bedside, the selection of the appropriate animal model is crucial. After all, the translatability of the results of the research depends on appropriate animal models that mimic the human condition. Li et al. [56] successfully developed a rhesus monkey model of PD by gene editing. For this, MRI-guided targeting of the PINK1 sites of the substantia nigra took place using a viral vector CRISPR/Cas9 system. This group of adult monkeys was susceptible to gene editing by showing a fast (6–10 months) process of PD progression with all the classic PD symptoms, including bradykinesia, tremor, and postural instability, accompanied by key pathological hallmarks of PD, such as severe nigral DA neuron loss and evident alpha-synuclein pathology. By contrast, a younger phenotype of their counterparts did develop PD but the symptoms did not show these severe characteristics. The researchers believe that these monkeys provide a practical transgenic model for future PD studies.

Gene Therapy

Modification of the gene expression of cells in a living organ is now possible with genetically modified viruses, called vectors. They infect cells without replicating or evoking its disease effects but can deliver therapeutic genes. Early preclinical studies in animal models of PD revealed that gene expression on the cells of an injured nigro-striatal pathway can counteract motor behavioral disturbances via different routes. Three main strategies are identified: (1)

modification of the motor circuitry like the effects of DBS, (2) introduction of enhanced expression of the machinery for DA synthesis to augment the therapeutic effect of the oral intake of levodopa DA precursor, and (3) counteracting the functional loss or increase survival of the nigral DA neurons and its fiber pathways. Buttery and Barker [57] recently reviewed the status of these approaches.

Mimicking DBS in the subthalamic nucleus is achieved using AAV-glutamic acid decarboxylase vectors introducing the local expression glutamic acid decarboxylase that increases the level of and chronic release of the inhibitory neurotransmitter gamma-aminobutyric acid. Results of a phase 1 study indicated that this therapy can be considered to be safe and well-tolerated in advanced PD, with no evidence of adverse effects or immunologic reactions [58]. One year after treatment, patients exhibited a statistically significant improvement of 27% in motor function on the side of their body correlating to the treated part of the brain with no improvement for the untreated side.

Augmenting the intrastriatal endogenic DA synthesis was attained by the application of an AAV-amino acid decarboxylase vector that induce the expression of L-amino acid decarboxylase that will subsequently boost the synthesis of DA from the orally taken levodopa. Positive effects in preclinical studies in Parkinsonian monkeys paved the way for this approach in patients [59]. In their survey of the many phase 1 studies in patients, Buttery and Barker [57] concluded that the method is well-tolerated that levodopa intake could be decreased with better results of motor scores in PD; however, placebo effects still have to be excluded.

Glial cell line-derived neurotrophic factor and neurturin are able to enhance survival and neurite outgrowth of DA neurons in vitro and in vivo and both can rescue DA neurons in preclinical models of PD. To this end, human trials on late phase PD patients with AAV and LV vectors, expressing these proteins within the striatal complex, hardly showed significant effect on the symptoms (reviewed by Buttery and Barker [57] and Axelsen and Woldbye [60]). The latter authors did not notice a significant effect during a controlled long-term infusion of glial cell line-derived neurotrophic factor (reviewed by Manfredsson et al. [61]). It is argued that the effect of these neurotrophic factors may evolve equal or better in the early stages of the disease. Intriguingly, the notion of early recognition of the presence of PD in humans can be done by smell [62].

Cell-Based Therapy

Following early observations in animal studies showing that immature neurons, i.e., neurons that still have to integrate into neuronal networks, can survive transplantation [63], the idea of replacement of lost or dysfunctioning human neurons started to evolve. The use of neuronal cell transplantation has been suggested to overcome neurodegenerative diseases, particularly when the disease can be pinpointed to a particular site or neuronal cell type. In fact, this therapy for PD was tested in various clinical trials [63]. During the 1990s, hundreds of patients in various centers worldwide received immature DA neurons derived from the human embryonic brain as grafts into their striata. However, subsequent double-blind, sham surgery, placebo-controlled studies showed variable and mostly negative results. They also revealed that some patients develop involuntary movements, so-called graft-induced dyskinesias, as a side effect. Meanwhile, treatment-induced pluripotent stem cells, primed in vitro to develop as DA neurons, seem to have found a pathway to proceed with further research and development [64, 65], despite ethical issues [66]. Notably, cell-based therapy for clinical use needs to be tested for viruses, biodistribution, (long term) toxicity, and tumorigenicity. Interestingly, Piao and co-workers [67] at Memorial Sloan Kettering Cancer Center, New York have generated midbrain dopamine neurons from human embryonic stem cells and manufactured large-scale cryopreserved DA progenitors for research and clinical use. A large-scale efficacy study in nude rats with induced hemi-Parkinsonism was performed; after stereotactic injection of cells at four deposits along a single track in the striatum, there were no obvious adverse effects, and the rescue of motor deficits were noticed at 4 and 6 weeks after application. Furthermore, after dissection of euthanized rats, no obvious distribution of cells outside the brain and no tumor formation were detected. The cells were manufactured under certified GMP conditions. Rigorous studies by Kirkeby and colleagues [68] at Lund, Sweden on in vivo cell survival and behavioral conditions in rats, as well as favorable GLP safety tests with regard to biodistribution, toxicity, and tumorigenicity in mice make it very likely that they are perfect for responsible clinical trials. To our knowledge, clinical trials with embryonic stem cells have been co-conducted by Dr. Tabar at Memorial Sloan Kettering Center, New York, NY, USA, (personal communication).

Conclusions

Many diseases are preventable by intervening before they are evident. Health risks can be reduced by avoiding bad habits, such as the use of tobacco, poor nutrition, and dangerous sports practices. Unfortunately, even after extensive research, the risk factors for PD are not fully established. Indeed, globally, PD is the fastest growing of all neurological disorders, with a prevalence of 6.1

million, which is projected to increase to over 12 million worldwide by 2050. Economically, the total US burden of PD was estimated to be USD 51.9 billion in 2017 [69]. The personal burden for caregivers has not been studied adequately, although a recent report mentions that people caring for patients with advanced PD had a greater perceived burden, were more likely to take medications, and had a lower treatment satisfaction compared to those caring for early or intermediate PD [70]. Possibly, some prevention can be obtained by avoiding indoor and outdoor pollution [71]. Although exposure to environmental factors like chemicals and toxins can modify the risk of PD, evidence for a causal relationship is insufficient [72].

In terms of disease modification, Belvisi et al. [73] suggested that antioxidant intake and physical activity slow the progression of PD. Until recently, there was little hope that disease progression or even reversion could be accomplished, but the studies of Belvisi et al. [73] and Ascherio et al. [74] brought fresh understanding that physical activity has beneficial effects on motor abnormalities in PD. Relevantly, fresh double-blind randomized studies regarding the benefit of targeted aerobic exercise on freezing gait have shown the efficacy on this disturbance and possibly other motor deficits [75–78]. The observed clinical improvement or even stabilization may well find its origin in diminished oxygenated stress, which is identified as one of the major contributors for neuron loss in both sporadic and genetic forms of PD [79]. This alteration in symptoms and hopefully disease modification supports our belief that the, likely long term, implementation of physical training in combination with pharmacological treatment may reduce the discomfort associated with this disabling disease.

As far as the genetic component is concerned, the Global Parkinson's Genetics Program, started in 2013, is of considerable importance for future research and development. The mission of this program "is to further understand the genetic architecture of PD through genotyping diverse participant groups and studying highly suspected monogenic forms of PD." As of August 4, 2022, 56 locations, 123 cohorts, with 160,308 samples expected and 8,644 samples completed illustrate the success of this global effort with 202 participating investigators. The program will be a valuable resource for the entire neurodegeneration community and will have a significant focus on training the next generation of PD researchers. Underlying data, analytical processes, and results from GP2 will be made available to the research community as quickly as possible, with minimal barriers to access and use.

In addition, we should not forget important developments like neurografting techniques using autologous DA cell type-primed precursor cells, which hold more promise than the previously used fetal mesencephalic neuron transplants [80]. Moreover, we should realize that there are CRISPR/Cas9 applications, in its original or modified forms. These hold much promise and offer many potential benefits. On the basis of achievements with CRISPR, it seems logical to start clinical trials in PD, as is already the case in treatment for many diseases [81, 82]. Notably, the monogenetic autosomal-dominant PD form with single mutations in the LRRK2 or SNCA gene, inherited from one parent would represent a good candidate for CRISPR/cas9 gene editing [55].

Last, but certainly not least, two very recent studies are worth mentioning and will have consequences for further preclinical and clinical studies. First, a study at Stanford University has defined a novel cellular pathway clearing misfolded proteins from the nucleus [83]. Misfolded proteins, like misfolded alpha-synuclein, are toxic to normal brain cells among other cells. By following the fate of the misfolded proteins through live-cell imaging and super-resolution microscopy, these researchers discovered that a pathway from the nucleus brings misfolded proteins to a vacuole full of degrading enzymes. Thus, the traffic of these proteins and their clearance inside the cellular degradation machinery reveals a promising study target for neurodegenerative diseases and therapies.

Studies on innovative molecular markers that possess diagnostic and prognostic potential are a continuous and challenging aspect of interest. In this respect, a breakthrough investigation by leading investigators may have direct clinical consequences. They have elaborated on an initial report by Kluge et al. [84] who isolated alpha-synuclein from PD patients and non-PD individuals and showed that the amplification of an alpha-synuclein seeding assay, called alpha-synuclein SAA, exhibited beta-sheet-rich structures and fibrillary appearance in blood samples from PD patients and not in the control group. Based on these results, Siderowf et al. [85] performed a cross-sectional study in patients enrolled during 2010–2019, analyzing the diagnostic performance of alpha-synuclein SAA in the spinal fluid of 545 PD patients, 163 healthy controls, 51 prodromal patients, and 310 non-manifesting LRRK or GBA carriers, using data from 33 participating academic centers worldwide. This recent study revealed that alpha-synuclein SAA classified people with PD with a high sensitivity of 87%

(95% CI: 84.9–90.5) and healthy controls with a high specificity of 96.3% (95% CI: 93.4–99.2). In the prodromal and high-risk groups, 86% (44 of 51 participants) had positive alpha-synuclein SAA. Non-manifesting LRRK2 or GBA carriers showed a positive test in 14 out of 159 (9%) and 11 out of 151 (7%) cases, respectively. Most importantly, this study demonstrates that this innovative biomarker can differentiate between people with PD from healthy controls with high sensitivity and specificity, paving the way to early diagnosis and treatment. According to the investigators there will be a crucial role for therapeutic development and the identification of at-risk cohorts. A further priority is to confirm whether the abovementioned blood (and not spinal fluid) test mentioned by Kluge et al. [84] has similar diagnostic reliability to classify individuals. Another recent study by Finnish researchers on PD biomarkers found that the Gram-negative sulfate-reducing bacteria of the genus Desulfovibrio play an important part in the genesis of PD [86, 87]. Desulfovibrio were reported that bacteria were present in the gut microbioma at higher levels in 20 PD patients than in twenty healthy controls. Additionally, the concentration of these bacterial species correlated with the clinically observed severity of PD ($p < 0.01$). Very recent Chinese research has confirmed these results [88], which suggests that Desulfovibrio bacteria take part in the pathogenesis of PD, which opens a new avenue for patient classification and treatment.

Acknowledgments

The authors are indebted to Mr. Richard B.Wolf for editorial assistance. The first author of this review would like to dedicate this paper to Professor Stig Hagen, emeritus neuropsychologist, with whom he had numerous discussions on the mystery of the brain.

Conflicts of Interest Statement

The authors have no conflicts of interest to disclose.

Funding Sources

No funding was provided.

Author Contributions

Ernest K. J. Pauwels composed the first and later drafts of the manuscript and prepared the final text. Gerard J. Boer reviewed the intermediate drafts, made alterations and additions, designed the images, and provided fresh data.

References

1 McGregor MM, Nelson AB. Circuit mechanisms of Parkinson's disease. Neuron. 2019;101(6):1042–56.
2 Brakedal B, Toker L, Haugarvoll K, Tzoulis C. A nationwide study of the incidence, prevalence and mortality of Parkinson's disease in the Norwegian population. NPJ Parkinsons Dis. 2022;8(1):19.
3 Kamath T, Abdulraouf A, Burris SJ, Langlieb J, Gazestani V, Nadaf NM, et al. Single-cell genomic profiling of human dopamine neurons identifies a population that selectively degenerates in Parkinson's disease. Nat Neurosci. 2022;25(5):588–95.
4 Spillantini MG, Schmidt M, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature. 1997;388(6645):839–40.
5 Shahnawaz M, Mukherjee A, Pritzkow S, Mendez N, Rabadia P, Liu X, et al. Discriminating α-synuclein strains in Parkinson's disease and multiple system atrophy. Nature. 2020;578(7794):273–7.
6 Sikora E, Bielak-Zmijewska A, Dudkowska M, Krzystyniak A, Mosieniak G, Wesierska M, et al. Cellular senescence in brain aging. Front Aging Neurosci. 2021;13:646924.
7 Chapman J, Fielder E, Passos JF. Mitochondrial dysfunction and cell senescence: deciphering a complex relationship. FEBS Lett. 2019;593(13):1566–79.
8 Vasileiou P, Evangelou K, Vlasis K, Fildisis G, Panayiotidis MI, Chronopoulos E, et al. Mitochondrial homeostasis and cellular senescence. Cells. 2019;8(7):686.
9 Velarde MC, Flynn JM, Day NU, Melov S, Campisi J. Mitochondrial oxidative stress caused by SOD2 deficiency promotes cellular senescence and aging phenotypes in the skin. Aging. 2012;4(1):3–12.
10 Bendor JT, Logan T, Edwards RH. The function of α-synuclein. Neuron. 2013;79(6):1044–66.
11 Shibasaki Y, Baillie DA, St Clair D, Brookes AJ. High-resolution mapping of SNCA encoding alpha-synuclein, the non-A beta component of Alzheimer's disease amyloid precursor, to human chromosome 4q21.3->q22 by fluorescence in situ hybridization. Cytogen Cellgen. 1995;71(1):54–5.
12 Gilmozzi V, Gentile G, Castelo Rueda MP, Hicks AA, Pramstaller PP, Zanon A, et al. Interaction of alpha-synuclein with lipids: mitochondrial cardiolipin as a critical player in the pathogenesis of Parkinson's disease. Front Neurosci. 2020;14:578993.
13 Schaser AJ, Osterberg VR, Dent SE, Stackhouse TL, Wakeham CM, Boutros SW, et al. Alpha-synuclein is a DNA binding protein that modulates DNA repair with implications for Lewy body disorders. Sci Rep. 2019;9(1):10919.
14 Provasek VE, Mitra J, Malojirao VH, Hegde ML. DNA double-strand breaks as pathogenic lesions in neurological disorders. Int J Mol Sci. 2022;23(9):4653.
15 Gcwensa NZ, Russell DL, Cowell RM, Volpicelli-Daley LA. Molecular mechanisms underlying synaptic and axon degeneration in Parkinson's disease. Front Cell Neurosci. 2021;15:626128.
16 Surmeier DJ. Determinants of dopaminergic neuron loss in Parkinson's disease. FEBS J. 2018;285(19):3657–68.
17 Power JH, Barnes OL, Chegini F. Lewy bodies and the mechanisms of neuronal cell death in Parkinson's disease and dementia with Lewy bodies. Brain Pathol. 2017;27(1):3–12.

18 Gonzalez-Rodriguez P, Zampese E, Surmeier DJ. Selective neuronal vulnerability in Parkinson's disease. Prog Brain Res. 2020;252:61–89.
19 Mahul-Mellier AL, Burtscher J, Maharjan N, Weerens L, Croisier M, Kuttler F, et al. The process of Lewy body formation, rather than simply α-synuclein fibrillization, is one of the major drivers of neurodegeneration. Proc Natl Acad Sci U S A. 2020 Mar 3;117(9):4971–82.
20 Volpicelli-Daley LA, Luk K, Lee VM. Addition of exogenous α-synuclein preformed fibrils to primary neuronal cultures to seed recruitment of endogenous α-synuclein to Lewy body and Lewy neurite-like aggregates. Nat Protoc. 2014;9:2135–46.
21 Fellner L, Gabassi E, Haybaeck J, Edenhofer F. Autophagy in α-Synucleinopathies-An Overstrained System. Cells. 2021;10(11):3143.
22 Kierdorf K, Prinz M. Factors regulating microglia activation. Front Cell Neurosci. 2013;7:44.
23 Zhang QS, Heng Y, Yuan YH, Chen NH. Pathological α-synuclein exacerbates the progression of Parkinson's disease through microglial activation. Toxicol Lett. 2017;265:30–7.
24 Han B, Jiang W, Cui P, Zheng K, Dang C, Wang J, et al. Microglial PGC-1α protects against ischemic brain injury by suppressing neuroinflammation. Genome Med. 2021;13(1):47.
25 Diaz-Castro B, Bernstein AM, Coppola G, Sofroniew MV, Khakh BS. Molecular and functional properties of cortical astrocytes during peripherally induced neuroinflammation. Cell Rep. 2021;36(6):109508.
26 Guttenplan KA, Weigel MK, Prakash P, Wijewardhane PR, Hasel P, Rufen-Blanchette U, et al. Neurotoxic reactive astrocytes induce cell death via saturated lipids. Nature. 2021;599(7883):102–7.
27 Zhang S, Shang D, Shi H, Teng W, Tian L. Function of astrocytes in neuroprotection and repair after ischemic stroke. Eur Neurol. 2021;84(6):426–34.
28 Joe EH, Choi DJ, An J, Eun JH, Jou I, Park S. Astrocytes, microglia, and Parkinson's disease. Exp Neurobiol. 2018;27(2):77–87.
29 Acioglu C, Li L, Elkabes S. Contribution of astrocytes to neuropathology of neurodegenerative diseases. Brain Res. 2021;1758:147291.
30 Herranz N, Gil J. Mechanisms and functions of cellular senescence. J Clin Invest. 2018;128(4):1238–46.
31 Song S, Lam EW, Tchkonia T, Kirkland JL, Sun Y. Senescent cells: emerging targets for human aging and age-related diseases. Trends Biochem Sci. 2020;45(7):578–92.
32 Yeh SH, Lin CH, Yang YJ, Lin LW, Tseng CW, Yang KD. Higher senescence associated secretory phenotype and lower defense mediator in urinary extracellular vesicles of elders with and without Parkinson disease. Sci Rep. 2021;11(1):15783.
33 Russo T, Riessland M. Age-related midbrain inflammation and senescence in Parkinson's disease. Front Aging Neurosci. 2022;14:917797.
34 Zhu Y, Tchkonia T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, et al. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell. 2015;14(4):644–58.
35 Moiseeva O, Deschênes-Simard X, St-Germain E, Igelmann S, Huot G, Cadar AE, et al. Metformin inhibits the senescence-associated secretory phenotype by interfering with IKK/NF-κB activation. Aging Cell. 2013;12(3):489–98.
36 Laberge RM, Zhou L, Sarantos MR, Rodier F, Freund A, de Keizer PL, et al. Glucocorticoids suppress selected components of the senescence-associated secretory phenotype. Aging Cell. 2012;11(4):569–78.
37 Karimi-Moghadam A, Charsouei S, Bell B, Jabalameli MR. Parkinson disease from Mendelian forms to genetic susceptibility: new molecular insights into the neurodegeneration process. Cell Mol Neurobiol. 2018;38(6):1153–78.
38 Blauwendraat CL, Heilbron K, Vallerga CL, Bandres-Ciga S, von Coelln R, Pihlstrøm L, et al. Parkinson's disease age at onset genome-wide association study: defining heritability, genetic loci, and α-synuclein mechanisms. Mov Disord. 2019;34(6):866–75.
39 Nuytemans K, Theuns J, Cruts M, Van Broeckhoven C. Genetic etiology of Parkinson disease associated with mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 genes: a mutation update. Hum Mut. 2010;31(7):763–80.
40 Funayama M, Nishioka K, Li Y, Hattori N. Molecular genetics of Parkinson's disease: contributions and global trends. J Hum Genet. 2023;68(3):125–30.
41 Yao S, Zhang X, Zou SC, Zhu Y, Li B, Kuang WP, et al. A transcriptome-wide association study identifies susceptibility genes for Parkinson's disease. NPJ Parkinsons Dis. 2021;7(1):79.
42 Markopoulou K, Chase BA, Premkumar AP, Schoneburg B, Kartha N, Wei J, et al. Variable effects of PD-risk associated SNPs and variants in Parkinsonism-associated genes on disease phenotype in a community-based cohort. Front Neurol. 2021;12:662278.
43 Srinivasan E, Chandrasekhar G, Chandrasekar P, Anbarasu K, Vickram AS, Karunakaran R, et al. Alpha-synuclein aggregation in Parkinson's disease. Front Med. 2021;8:736978.
44 Gadhe L, Sakunthala A, Mukherjee S, Gahlot N, Bera R, Sawner AS, et al. Intermediates of α-synuclein aggregation: implications in Parkinson's disease pathogenesis. Biophys Chem. 2022;281:106736.
45 Mao H, Ye Y, Sun X, Qian C, Wang B, Xie L, et al. Quiescent elongation of α-synuclein pre-form fibrils under different solution conditions. Front Neurosci. 2022;16:902077.
46 Hauser RA, Giladi N, Poewe W, Brotchie J, Friedman H, Oren S, et al. P2B001 (extended release pramipexole and rasagiline): a new treatment option in development for Parkinson's disease. Adv Ther. 2022;39(5):1881–94.
47 Antonini A, Pahwa R, Odin P, Isaacson SH, Merola A, Wang L, et al. Comparative effectiveness of device-aided therapies on quality of life and off-time in advanced Parkinson's disease: a systematic review and Bayesian network meta-analysis. CNS Drugs. 2022;36(12):1269–83.
48 Vitek JL, Jain R, Chen L, Tröster AI, Schrock LE, House PA, et al. Subthalamic nucleus deep brain stimulation with a multiple independent constant current-controlled device in Parkinson's disease (INTREPID): a multicentre, double-blind, randomised, sham-controlled study. Lancet Neurol. 2020;19(6):491–501.
49 Bove F, Mulas D, Cavallieri F, Castrioto A, Chabardès S, Meoni S, et al. Long-term outcomes (15 years) after subthalamic nucleus deep brain stimulation in patients with Parkinson disease. Neurology. 2021;97(3):e254–62.
50 Gilbert F, Lancelot M. Incoming ethical issues for deep brain stimulation: when long-term treatment leads to a 'new form of the disease'. J Med Ethics. 2021;47(1):20–5.
51 Chaib S, Tchkonia T, Kirkland J. Cellular senescence and senolytics: the path to the clinic. Nat Med. 2022;28(8):1556–68.
52 Hickson LJ, Langhi Prata LG, Bobart SA, Evans TK, Giorgadze N, Hashmi SK, et al. Senolytics decrease senescent cells in humans: preliminary report from a clinical trial of Dasatinib plus Quercetin in individuals with diabetic kidney disease. EBioMedicine. 2019;47:446–56.
53 Cummins N, Gotz J. Shedding light on mitophagy in neurons: what is the evidence for PINK1/Parkin mitophagy in vivo? Cell Mol Life Sci. 2018;75(7):1151–62.
54 Sun Z, Ye J, Yuan J. PINK1 mediates neuronal survival in monkey. Protein Cell. 2022;13(1):4–5.
55 Rahman MU, Bilal M, Shah JA, Kaushik A, Teissedre PL, Kujawska M. CRISPR-Cas9-based technology and its relevance to gene editing in Parkinson's disease. Pharmaceutics. 2022;14(6):1252.
56 Li H, Wu S, Ma X, Li X, Cheng T, Chen Z, et al. Co-editing*PINK1* and *DJ-1* Genes via adeno-associated virus-delivered CRISPR/Cas9 system in adult monkey brain elicits classical Parkinsonian phenotype. Neurosci Bull. 2021;37(9):1271–88.
57 Buttery PC, Barker RA. Gene and cell-based therapies for Parkinson's disease: where are we? Neurotherapeutics. 2020;17(4):1539–62.

58 Feigin A, Kaplitt MG, Tang C, Lin T, Mattis P, Dhawan V, et al. Modulation of metabolic brain networks after subthalamic gene therapy for Parkinson's disease. Proc Natl Acad Sci USA. 2007;104(49):19559–64.
59 Bankiewicz KS, Forsayeth J, Eberling JL, Sanchez-Pernaute R, Pivirotto P, Bringas J, et al. Long-term clinical improvement in MPTP-lesioned primates after gene therapy with AAV-hAADC. Mol Ther. 2006;14(4):564–70.
60 Axelsen TM, Woldbye DPD. Gene therapy for Parkinson's disease, an update. J Parkinsons Dis. 2018;8(2):195–215.
61 Manfredsson FP, Polinski NK, Subramanian T, Boulis N, Wakeman DR, Mandel RJ. The future of GDNF in Parkinson's disease. Front Aging Neurosci. 2020 Dec 7;12:593572.
62 Sarkar D, Sinclair E, Hway Lim S, Walton C, Jafri K, Milne J, et al. Paper spray ionization ion mobility mass spectrometry of sebum classifies biomarker classes for the diagnosis of Parkinson's d. JACS Au. 2022 Sep 7;2(9):2013–2022.
63 Bjorklund A, Stenevi U. Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. Brain Res. 1979;177(3):555–60.
64 Hiller BM, Marmion DJ, Thompson CA, Elliott NA, Federoff H, Brundin P, et al. Optimizing maturity and dose of iPSC-derived dopamine progenitor cell therapy for Parkinson's disease. NPJ Regen Med. 2022;7(1):24.
65 Kirkeby A, Parmar M, Barker RA. Strategies for bringing stem cell-derived dopamine neurons to the clinic: a European approach (STEM-PD). Prog Brain Res. 2017;230:165–90.
66 Boer GJ. Ethical issues in neurografting of human embryonic cells. Theor Med Bioeth. 1999;20(5):461–75.
67 Piao J, Zabierowski Pickrell AM, Youle RJ. The roles of PINK1, Parkin, and mitochondrial fidelity in Parkinson's disease. Neuron. 2015;85:0896–6273.
68 Tomishima M, Kirkeby A. Bringing advanced therapies for Parkinson's disease to the clinic: the scientist's perspective. J Parkinsons Dis. 2021;11(s2):S135–S140.
69 Nandipati S, Litvan I. Environmental exposures and Parkinson's disease. Int J Environ Res Public Health. 2016;13(9):881.
70 Bjorklund G, Stejskal V, Urbina MA, Dadar M, Chirumbolo S, Mutter J. Metals and Parkinson's disease: mechanisms and biochemical processes. Curr Med Chem. 2018;25(19):2198–214.
71 Yang W, Hamilton JL, Kopil C, Beck JC, Tanner CM, Albin RL, et al. Current and projected future economic burden of Parkinson's disease in the U.S. NPJ Parkinsons Dis. 2020;6:15.
72 Martinez-Martin P, Skorvanek M, Henriksen T, Lindvall S, Domingos J, Alobaida A, et al. J Neurol. Impact of advanced Parkinson's disease on caregivers: an international real-world study. J Neurol. 2023:1–12.
73 Belvisi D, Pellicciari R, Fabbrini G, Tinazzi M, Berardelli A, Defazio G Neurobiol Dis. 2020;134:104671.
74 Hughes KC, Gao X, Molsberry S, Valeri L, Schwarzschild MA, Ascherio A. Physical activity and prodromal features of Parkinson disease. Neurology. 2019;93(23):e2157–69.
75 Ellis T, Rochester L. Mobilizing Parkinson's disease: the future of exercise. J Parkinsons Dis. 2018;8(s1):S95–100.
76 van der Kolk NM, de Vries NM, Kessels RPC, Joosten H, Zwinderman AH, Post B, et al. Effectiveness of home-based and remotely supervised aerobic exercise in Parkinson's disease: a double-blind, randomised controlled trial. Lancet Neurol. 2019;18(11):998–1008.
77 Gilat M, Ginis P, Zoetewei D, De Vleeschhauwer J, Hulzinga F, D'Cruz N, et al. A systematic review on exercise and training-based interventions for freezing of gait in Parkinson's disease. NPJ Parkinsons Dis. 2021;7(1):81.
78 Li F, Wang D, Ba X, Liu Z, Zhang M. The comparative effects of exercise type on motor function of patients with Parkinson's disease: a three-arm randomized trial. Front Hum Neurosci. 2022;16:1033289.
79 Ammar A, Trabelsi K, Boukhris O, Glenn JM, Bott N, Masmoudi L, et al. Effects of aerobic-anaerobic- and combined-based exercises on plasma oxidative stress biomarkers in healthy untrained young adults. Int J Environ Res Public Health. 2020;17(7):2601.
80 Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow W. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. Nat Med. 2008;14(5):504–6.
81 Arabi F, Mansouri V, Ahmadbeigi N. Gene therapy clinical trials, where do we go? An overview. Biomed Pharmacother. 2022;153:113324.
82 De Plano LM, Calabrese G, Conoci S, Guglielmino SPP, Oddo S, Caccamo A. Applications of CRISPR-Cas9 in Alzheimer's disease and related disorders. Int J Mol Sci. 2022;23(15):8714.
83 Sontag EM, Morales-Polanco F, Chen JH, McDermott G, Dolan PT, Gestaut D, et al. Nuclear and cytoplasmic spatial protein quality control is coordinated by nuclear-vacuolar junctions and perinuclear ESCRT. Nat Cell Biol. 2023;25(5):699–713.
84 Kluge A, Bunk J, Schaeffer E, Drobny A, Xiang W, Knacke H, et al. Detection of neuron-derived pathological α-synuclein in blood. Brain. 2022 Sep 14;145(9):3058–71.
85 Siderowf A, Concha-Marambio L, Lafontant DE, Farris CM, Ma Y, Urenia PA, et al. Assessment of heterogeneity among participants in the Parkinson's Progression Markers Initiative cohort using α-synuclein seed amplification: a cross-sectional study. Lancet Neurol. 2023;22(5):407–17.
86 Murros KE, Huynh VA, Takala TM, Saris PEJ. *Desulfovibrio* Bacteria Are Associated With Parkinson's Disease.. Front Cell Infect Microbiol. 2021;11:652617.
87 Huynh VA, Takala TM, Murros KE, Diwedi B, Saris PEJ. Desulfovibrio bacteria enhance alpha-synuclein aggregation in a Caenorhabditis elegans model of Parkinson's disease. Front Cell Infect Microbiol. 2023, May 1.
88 Nie S, Jing Z, Wang J, Deng Y, Zhang Y, Ye Z, Ge Y. The link between increased Desulfovibrio and disease severity in Parkinson's disease. Appl Microbiol Biotechnol. 2023;107(9):3033–3045.