Parkinson's Disease 2

The pathogenesis of Parkinson's disease

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Parkinson's disease is a progressive neurodegenerative condition associated with the deposition of aggregated α-synuclein. Insights into the pathogenesis of Parkinson's disease have been derived from genetics and molecular pathology. Biochemical studies, investigation of transplanted neurons in patients with Parkinson's disease, and cell and animal model studies suggest that abnormal aggregation of α-synuclein and spreading of pathology between the gut, brainstem, and higher brain regions probably underlie the development and progression of Parkinson's disease. At a cellular level, abnormal mitochondrial, lysosomal, and endosomal function can be identified in both monogenic and sporadic Parkinson's disease, suggesting multiple potential treatment approaches. Recent work has also highlighted maladaptive immune and inflammatory responses, possibly triggered in the gut, that accelerate the pathogenesis of Parkinson's disease. Although there are currently no disease-modifying treatments for Parkinson's disease, we now have a solid basis for the development of rational neuroprotective therapies that we hope will halt the progression of this disabling neurological condition.

Introduction

Parkinson's disease is a common neurodegenerative disorder that causes major disability and an increasing global public health burden related to motor, non-motor, and cognitive features.[1] Advances in the genetics of Parkinson's disease, beginning with the identification of α-synuclein as the first autosomal dominant gene for Parkinson's disease, have led to a rapid increase in our understanding of its pathogenesis. The major challenges of understanding the disease include identification of new pathways in the development and progression of the disease, and the correlation of these mechanisms with the heterogeneous clinical manifestations and disease course. A series of mechanism-based Parkinson's disease-modifying trials are planned or in progress, directly related to these advances.[2] Some trials are now selecting patients with specific genetic variants, and future therapies will possibly be targeted to disease mechanisms in a stratified medicine approach based on biomarkers and genotype. This Series paper summarises recent advances in the understanding of the pathogenesis of Parkinson's disease and highlights probable future developments.

Clinical aspects of Parkinson's disease

Parkinson's disease is a clinicopathological syndrome in which progressive asymmetric slowness of movement (bradykinesia), rigidity, tremor, and gait disturbance are associated with neuronal loss and the formation of α-synuclein-containing proteinaceous aggregates in neurons of the substantia nigra, known as Lewy bodies and Lewy neurites.[3,4] Parkinsonism might have diverse pathological underlying causes, including tau, polyglutamine, and Alzheimer's disease pathology, as well as nigral cell loss without hallmark pathological features.[5–7] Several of these causes of non-Lewy body parkinsonism have been reported in populations of non-European ancestry.[6,8–10] Lewy body Parkinson's disease is the primary focus of this Series paper, but a broader conception of the pathogenesis of parkinsonism is possibly needed to describe the relevant disease mechanisms in different parts of the world.

The definition of Parkinson's disease in the Queen Square Brain Bank clinical diagnostic criteria primarily relates to the levodopa-responsive motor phenotype, and directly relates to cell loss in the substantia nigra and dopaminergic denervation of the caudate and putamen.[4] Although the earliest descriptions of Parkinson's disease included non-motor features, clinicopathological work over the past 20 years has focused on the involvement of neural systems that affect the gut, autonomic nervous system, sleep, smell, anxiety, and cognition. The Movement Disorder Society clinical diagnostic criteria for Parkinson's disease include weighting for these non-motor features, which are often helpful in reaching a clinical diagnosis, and are recognised as central determinants of impaired quality of life and morbidity for patients with Parkinson's disease.[11] Parkinson's disease non-motor features might also provide important clues as to the underlying disease pathogenesis.

Parkinson's disease is a progressive condition, and studies of disease pathogenesis encompass both the initiation of the disease and the development of

Search strategy and selection criteria

We searched PubMed for review articles published in English between July 11, 2017, and June 26, 2023 that included "Parkinson's" with "pathogenesis" OR "genetics" OR "aetiology" OR "biochemistry" OR "pathology". We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant, supplemented by original articles provided by the authors. Review articles are cited to provide readers with an overview of some aspects of the pathogenesis of Parkinson's disease, within the space constraints of this Series paper.

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This is the second in a **Series** of three papers about Parkinson's disease. All papers in the Series are available at www.thelancet.com/series/parkinsons-disease

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progressively worsening clinical features, with the involvement of multiple brain regions. Here we will review the pathogenesis of Parkinson's disease in relation to recent developments in genetics, neuropathology, and cellular mechanisms, and highlight the development of new biomarkers and emerging routes to new therapies (table).

Genetics

Parkinson's disease is a genetic disorder in the narrow sense that some patients have well defined causal rare variants leading to familial disease, and in the broad sense that many patients have polygenic risk for disease based on a series of common risk variants.[12] The heritability of Parkinson's disease has been estimated from both twin studies and statistical genetic methods to lie between 22% and 40%, so the cause of Parkinson's disease is likely to have a substantial genetic and environmental component.[1] Three well validated autosomal dominant genes (*SNCA*, *LRRK2*, and *VPS35*) and three well validated autosomal recessive genes (*PRKN*, *PINK1*, and *DJ1* [also known as *PARK7*]) are known to cause Parkinson's disease, together with a series of genes that have been reported in small numbers of cases or families. In addition, common and single rare variants in the Gaucher disease-causing gene *GBA1*, which encodes glucocerebrosidase, are associated with Parkinson's disease, but usually do not segregate in autosomal dominant families.[13] Numerically, *LRRK2* is the most important mendelian cause of Parkinson's disease, with the Gly2019Ser mutation occurring most commonly in European, North African, and Jewish kindreds, Asn1437Asp mutation in Chinese kindreds, and Ile2020Thr in Japanese kindreds.[14] Additionally, the *LRRK2* Gly2385Arg variant is a risk factor for Parkinson's disease in the Chinese and Korean populations with an odds ratio of approximately 2·4.[15] *LRRK2* is also implicated in the development of Crohn's disease, leprosy, and mycobacterial disease, and there might be pleiotropic effects at the *LRRK2* locus regulating susceptibility and resistance to infectious and inflammatory disease and neurodegeneration.[14]

SNCA mutations are a rarer cause of autosomal dominant Parkinson's disease. The identification of the Ala53Thr mutation in the *SNCA* gene in an Italian American family with autosomal dominant Parkinson's disease led to the identification of α-synuclein protein as the hallmark component of Lewy bodies and Lewy neurites in Parkinson's disease and of glial cytoplasmic inclusions in multiple system atrophy.[16,17] The identification of pathogenic *SNCA* gene multiplications

	Proposed pathogenesis	Genetic evidence	Biomarkers	Therapeutic implications
Increase in *SNCA* expression	Increase in α-synuclein protein leads to increased aggregation and cell death and dysfunction	Increased *SNCA* gene dose (duplications or triplications) causes Parkinson's disease; *SNCA* common variants probably lead to increased expression	α-Synuclein and phospho-synuclein measurement in blood and CSF	Decrease in *SNCA* transcription or translation (eg, with ASO therapy)
Increase in α-synuclein aggregation	Formation of oligomers and fibrils leads to cellular toxicity	Coding mutations in *SNCA* lead to increase in α-synuclein aggregation	rt-QUIC assays for aggregation based on CSF, skin biopsies, olfactory mucosal biopsies, and saliva	Antiaggregation therapies
Mitochondrial dysfunction	Reduced complex 1 activity, abnormal calcium homoeostasis, increased reactive oxygen species, and reduced mitochondrial ATP production	Multiple Parkinson's disease gene mutations lead to changes in mitochondrial function including *PRKN*, *PINK1*, and *LRRK2*	Magnetic resonance spectroscopy analysis of Pi to ATP ratios, measurement of ATP, and mitochondrial function in skin fibroblasts	Enhancing mitochondrial biogenesis and function
Altered endosomal-lysosomal trafficking	Activation of LRRK2 and VPS35 lead to phosphorylation of Rab proteins, which leads to decreased lysosomal function and altered response to membrane damage	Rare pathogenic variants in *LRRK2* (eg, Gly2019Ser) and *VPS35* lead to increased Rab phosphorylation	Measurement of Rab protein phosphorylation in cells from peripheral blood; measurement of urinary BMP phospholipids	Reducing LRRK2 protein levels, or kinase activity, or both, with ASO therapy or kinase inhibitors
Lysosomal dysfunction	Impaired α-synuclein degradation leads to increased cellular α-synuclein	*GBA1* mutations are associated with Parkinson's disease, and rare variants in other genes might be relevant	Measurement of GCase protein and enzyme activity; measurement of GSLs in blood and CSF; measurement of urinary BMP phospholipids	Modulators of GCase activity
Immune activation and neuroinflammation	Multiple factors (α-synuclein aggregates, mitochondrial antigens, and gut bacterial endotoxins) promote both innate and adaptive immune responses, culminating in increased neuroinflammation and neuronal toxicity	Association between HLA variants and Parkinson's disease; LRRK2, PRKN, and PINK1 are involved in inflammatory pathway	Measurement of C-reactive protein, interleukins, and PET imaging of activated microglia	Immunomodulatory or anti-inflammatory therapies
Cell-to-cell spread	Toxic forms of α-synuclein spread between anatomically contiguous cells, or over longer range, and might be contained in extracellular vesicles	NA	rt-QUIC assays for aggregation based on CSF, skin biopsies, olfactory mucosal biopsies, and saliva	Reduction in release, extracellular transit, or uptake by recipient cells using monoclonal antibody therapy or other therapies

ASO=antisense oligonucleotide. BMP=bis(monoacylglycerol)phosphate. CSF=cerebrospinal fluid. GCase=β-glucocerebrosidase. GSLs=glycosphingolipids. NA=not applicable. rt-QUIC=real time quaking-induced conversion.

Table: **Proposed pathogenic mechanisms in Parkinson's disease**

showed that an increase in the level of production of SNCA mRNA and α-synuclein could be sufficient to cause Parkinson's disease.[18] Many families with *SNCA* mutations have prominent cortical Lewy body formation, autonomic failure, and pathology that overlaps between multiple system atrophy, dementia with Lewy bodies, and Parkinson's disease, indicating shared mechanisms and clinical features across these disorders. These patients might also have early disease onset and rapid progression to poor outcomes, indicating that *SNCA*-related mechanisms might be a driver of disease severity.

Genome-wide association studies have been used to investigate differences in common variation across the genome in the susceptibility to the development of Parkinson's disease. To date, 90 independent variants across 74 genomic loci have been associated with disease risk, each conferring a small increase in disease risk with an odds ratio of less than 2.[12] The strongest genome-wide association study associations with Parkinson's disease in European populations are at the *SNCA* and *MAPT* loci. Recently, common variation at the *GBA1* locus has been defined as the major risk for Parkinson's disease in populations with African ancestry.[19] Although *MAPT* encodes the microtubule-associated protein tau, tau pathology is not a ubiquitous feature of Parkinson's disease pathology, and the Parkinson's disease causal genes at the *MAPT* locus remain poorly understood. Evidence supports the role of a gene-regulating mitochondrial function, *KANSL1* at the *MAPT* locus.[20] Annotation of functional pathways from these loci supports the involvement of the lysosomal autophagy system and immune inflammatory mechanisms in the pathogenesis of Parkinson's disease.[21,22] Most genome-wide association studies have focused on disease risk (using a case-control design) in European ancestry populations. There is a pressing need to understand more about the biology of disease variation and the genetic basis of disease in non-European populations, which is under investigation in international consortia, including the Global Parkinson's Genetics Program.

Neuropathology

Clinicopathological correlation was used to define the nosology of Parkinson's disease in the past century, and more recent advances in structural and biochemical pathology in humans and model systems have led to advances in our understanding of the pathogenesis. Lewy bodies and Lewy neurites, first described by Frederic Lewy in 1912, contain the protein α-synuclein, which forms filaments that trap organelles such as mitochondria and lysosomes.[23–26] Pathogenic mutations in α-synuclein lead to accelerated aggregate formation, suggesting that α-synuclein dysfunction is an early step in familial, and probably sporadic, Parkinson's disease (figure 1). The presence of pathological α-synuclein aggregates at synapses in animal model and human neuropathology studies suggests that early synaptic dysfunction might be an important step in the pathogenesis of Parkinson's disease.[27,28] Recently, cryo-electron microscopy studies have shown that α-synuclein filament structure is similar in Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies, but different in multiple system atrophy.[25,29]

α-Synuclein is a 140-amino acid protein that contains tandem repeats in the amino terminal part, which can bind lipid membranes. α-Synuclein is abundant in the brain, where it is found in neurons (particularly in synaptic terminals) and is involved in vesicle transport and neurotransmitter release.[29] Small oligomeric aggregates are also present in blood and cerebrospinal fluid.[30] Lewy bodies represent the end stage of a cellular process, in which initial small α-synuclein aggregates are observed in the cytoplasm of the neuron and then coalesce into diffuse pale bodies. This coalescence is followed by the formation of an aggregation seed, which leads to diffuse synuclein aggregating into filaments. These filaments then form a classic Lewy body (figure 1).[31] Lewy bodies might be detrimental to neurons in that they are space occupying lesions that might alter cellular function; however, most evidence supports the toxicity of small aggregates and oligomers in the pathogenesis of Parkinson's disease, rather than larger aggregates.[32]

Using α-synuclein staining to detect Lewy bodies in post-mortem donors with differing levels of Lewy body pathology, Braak and colleagues proposed a staging of Parkinson's disease, in which the disease could start in the periphery, in the gut, or in the olfactory system, and spread to subcortical and then cortical brain regions (figure 1).[31] The Braak hypothesis is supported by studies of the prodromal phases of Parkinson's disease, including unbiased studies of primary health-care records, which show that in the general population, people who develop

For more on the **Global Parkinson's Genetics Program** see gp2.org.

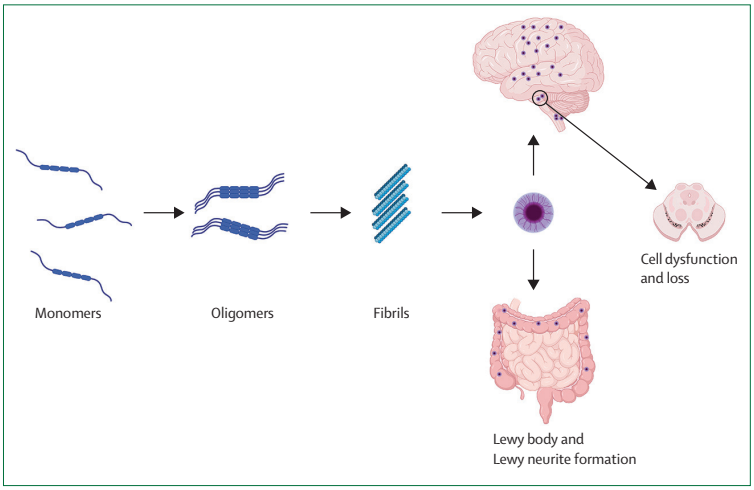

Figure 1: **α-Synuclein has a central role in the pathophysiology of Parkinson's disease**
α-Synuclein aggregates in oligomers and fibrils, and forms Lewy bodies found in the gut, brainstem, and cerebral cortex

motor Parkinson's disease have higher rates of multiple non-motor features, including constipation, depression, anxiety, hypotension, urinary dysfunction, and erectile dysfunction.[33] These data have been interpreted as supporting the presence of Parkinson's disease pathology in non-motor regions, including the peripheral autonomic nervous system, dorsal motor nucleus of the vagus nerve, and olfactory system, before the development of motor disease; however, although the Braak hypothesis is useful in categorising Lewy body pathology in Parkinson's disease, many Parkinson's disease cases do not follow the Braak neuropathological staging system.[34] Clinical and neuroimaging studies have suggested that some patients might have brainstem-predominant disease without evidence of peripheral pathology.[35] Furthermore, some patients might have olfactory involvement without brainstem involvement, and then either brainstem-predominant or limbic-predominant disease. This diversity in Lewy body distribution was described in a revised 2009 unified Lewy body staging system.[36]

In 2008, the concept of α-synuclein spread in the nervous system was supported by direct neuropathological evidence. Clinical trials had been done in which the dopaminergic deficit in some patients with Parkinson's disease was treated by transplanting human fetal midbrain neurons.[37,38] Autopsies from several graft recipients in these trials showed evidence for Lewy body formation within the intrastriatal transplanted fetal midbrain neurons.[37,38] This evidence strongly suggested that these cells had developed Lewy bodies through spread from the Lewy pathology in the surrounding brain.

The hypothesis of the spreading of α-synuclein pathology has been validated using several models, including injection of preformed α-synuclein fibrils into the brain, and injection of adeno-associated viruses expressing high amounts of α-synuclein, leading to its aggregation (figure 1). These spreading studies have been performed in mice, rats, and non-human primates, and support both the spread of α-synuclein from the periphery to the CNS and from the brain to other organs.[39–42] Pathological spread is supported by the occurrence of premotor gastrointestinal symptoms that might precede the substantia nigra-related motor symptoms by decades.[33,43] Specifically, the presence of α-synuclein aggregates in neurons surrounding the gut could be the cause of the constipation that has been described in around 20% of patients before onset of motor symptoms. Rapid eye movement (REM) sleep behaviour disorder is thought to appear when the α-synuclein aggregates reach the locus coeruleus and thalamus; development of REM sleep behaviour disorder is associated with the appearance of a movement disorder within the following 10 years.[44]

Molecular mechanisms contributing to Parkinson's disease

Identification of genes that are altered in genetic forms of Parkinson's disease has provided a greater understanding of the molecular mechanisms that contribute to pathobiology.[45] Disruption of interorganellar homoeostasis, impaired mitochondrial and lysosomal function, altered lipid metabolism, endoplasmic reticulum stress, and defective signalling between the endoplasmic reticulum and mitochondria result in a cascade of events associated with the accumulation of α-synuclein,[46,47] deposition of oligomers and fibrils, and formation of Lewy bodies and Lewy neurites, which cause synaptic dysfunction and neurodegeneration (figure 2).

Mitochondrial mechanisms

Mitochondria have a fundamental role in the neurodegenerative process of Parkinson's disease. They have key roles in cellular energy production and cell signalling processes that use the cell's bioenergetic status to determine whether the cell survives or whether it undergoes degeneration.[48] Synaptic damage and mitochondrial dysfunction are early events in the pathogenesis of Parkinson's disease, and alterations of mitochondrial structure and dynamics are linked to increased production of reactive oxygen species, abnormal intracellular calcium levels, and reduced mitochondrial ATP production (figure 2).[49]

Both genetic and environmental factors have been associated with mitochondrial dysfunction in Parkinson's disease pathogenesis. The consistent epidemiological evidence linking pesticide exposure to the risk of Parkinson's disease and the report of a cluster of patients with drug-induced parkinsonism related to MPTP toxicity implicate specific toxins in nigral damage, which mediate their effect through mitochondrial complex I inhibition.[50,1] The use of neurotoxins to inhibit mitochondrial complex I and induce Parkinson's disease-like syndromes in animal models has provided mechanistic evidence linking mitochondrial dysfunction to Parkinson's disease. A decrease of mitochondrial complex I activity has been reported in patients with Parkinson's disease and in neurotoxin-induced or genetic factor-induced in vitro and in vivo models.[51] Insights from genetic studies have shown that mutations in 11 genes that can cause parkinsonian syndromes (*SNCA, PRKN, PINK1, DJ1, LRRK2, ATP13A2, PLA2G6, FBXO7, VPS35, CHCHD2,* and *VPS13C*) alter mitochondrial energy production, reactive oxygen species production, mitochondrial biogenesis, and quality control, underpinning a central role for mitochondria in Parkinson's disease.[52]

Neurons (particularly dopaminergic neurons) have high energy requirements, necessitating high-quality mitochondrial bioenergetic function for the normal function and survival of the cells.[53,54] Mitophagy, a process that selectively targets damaged or redundant mitochondria to the lysosome for elimination via the autophagic pathways, is crucial in preserving mitochondrial health.[55] Two Parkinson's disease genes, *PINK1* and *PRKN*, are involved in mitochondrial quality control in response to overt mitochondrial stress, but are not essential for all types of mitophagy. The *LRRK2* Gly2019Ser mutation, the most

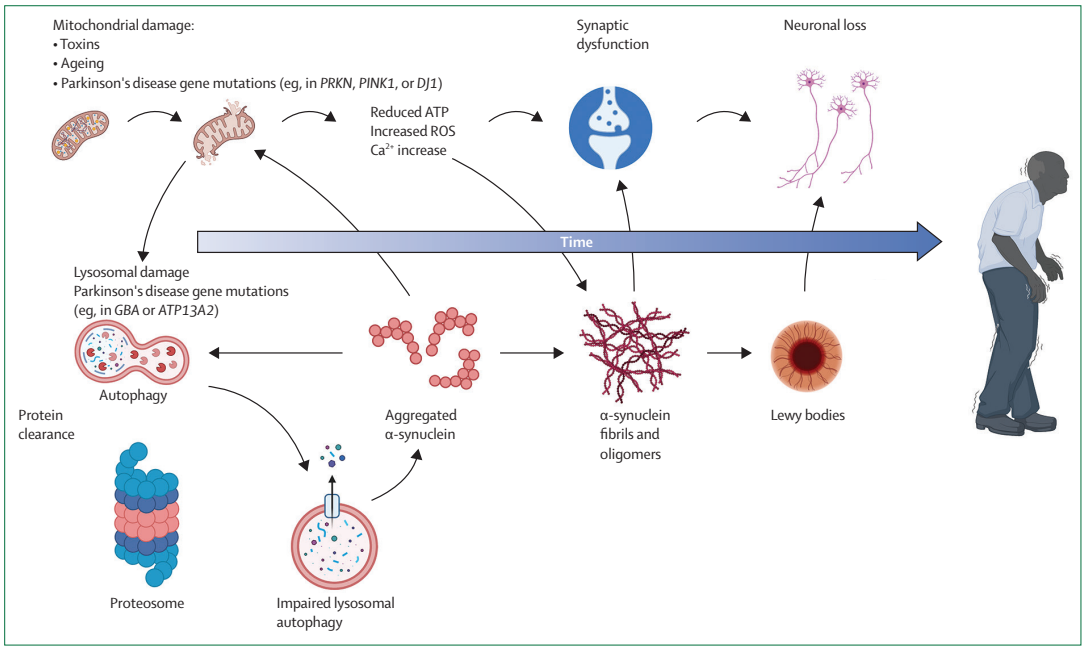

Figure 2: **Molecular mechanisms contributing to Parkinson's disease**
ATP=adenosine triphosphate. ROS=reactive oxygen species.

common *LRRK2* mutation in Parkinson's disease, was recently shown to disrupt basal mitophagy in vivo, indicating that other forms of mitophagy might have a role in neuronal survival.[56] Emerging literature indicates that other mitochondrial interactions contribute to the cause of Parkinson's disease. For example, aggregated α-synuclein has been reported to permeabilise through the mitochondrial membrane and impair the electron transport chain to enhance oxidative stress-mediated apoptosis in neurons, and this process might be further exacerbated by hypoxia.[57,58] Mitochondrial dysfunction and the overproduction of reactive oxygen species facilitate the formation of soluble α-synuclein oligomers and insoluble fibrils.[59]

Proteostasis and lysosomal dysfunction

The autophagy-lysosomal system and the ubiquitin-proteasome system mediate the selective and targeted degradation of abnormal or misfolded protein species. In Parkinson's disease, mitochondrial dysfunction and a decline in the clearance capacity of the ubiquitin-proteasome and autophagy-lysosomal systems have been implicated in the pathobiology of Parkinson's disease (figure 2).

Lysosomes are involved in autophagy and mitophagy, and provide pathways to clear abnormal or accumulated proteins.[60] α-Synuclein degradation is mostly lysosomal dependent, and lysosomal impairment can affect α-synuclein turnover, causing an increase in its cellular levels and subsequent aggregation.[61]

Insights from genes associated with lysosomal function are mainly provided by mutations in *GBA1*. Heterozygous mutations in *GBA1* have been proposed to cause a deficiency of the lysosomal enzyme acid β-glucocerebrosidase (GCase). GCase has been shown to have a bidirectional relationship with α-synuclein, resulting in a pathogenic feedback loop that can lead to progressive α-synuclein accumulation.[62] Reduced GCase activity leads to the accumulation of glucosylceramide (GlcCer), which is deacylated by lysosomal acid ceramidase to a toxic metabolite, glucosylsphingosine, and subsequent activation of astrocytes and microglia, releasing pro-inflammatory mediators and causing extensive neuroinflammation.[63,64] The association between *GBA1*, lysosomal mechanisms, and Parkinson's disease is not straightforward, given that non-Gaucher disease-causing mutations are also associated with Parkinson's disease, and a recent trial of substrate reduction with venglustat (Sanofi, Paris, France), which successfully lowered the levels of GlcCer, had no effect on Parkinson's disease progression.[65]

Endocytosis and cellular trafficking

The involvement of *LRRK2* and *VPS35* in the development of Parkinson's disease implicates abnormalities of endocytosis and intracellular trafficking. Endocytosis and the formation of the early endosome is followed by recycling to the membrane, retrograde transport to the trans-Golgi network, or endosomal maturation through the endosomal sorting complexes required for transport

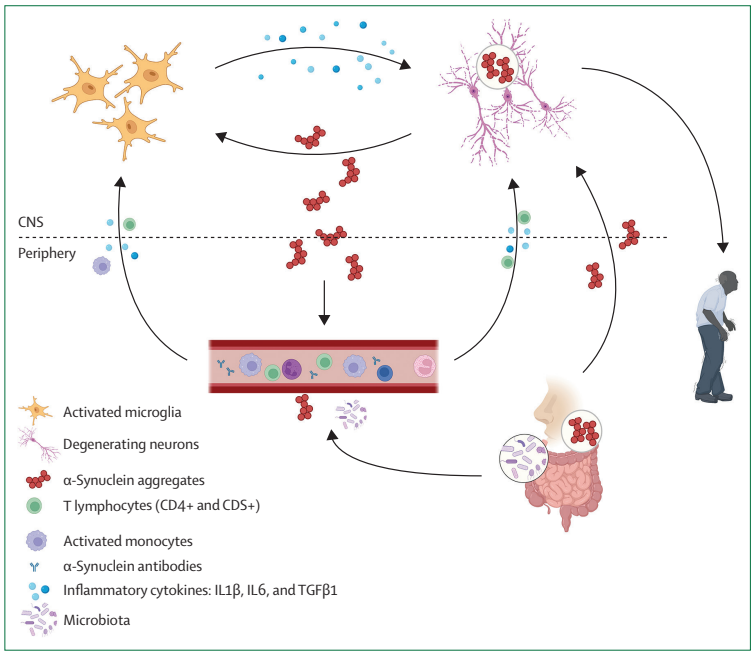

Figure 3: **Proposed peripheral–central immune interactions in the pathogenesis of Parkinson's disease**

pathway, leading to lysosomal fusion and degradation.[66] LRRK2 is a protein kinase, and pathogenic mutations increase the phosphorylation of a series of Rab proteins including RAB10, RAB12, and RAB29. Rab proteins are involved in endocytosis and lysosomal trafficking, and the end result of LRRK2 overactivation might be a deficiency in lysosomal function or aberrant cellular response to membrane damage, or both.[14] Pathogenic *VPS35* mutations also lead to activation of LRRK2, which has also been reported in sporadic (non-monogenic) Parkinson's disease, suggesting that LRRK2 activation might be a common pathway in Parkinson's disease pathogenesis.[66,67]

Immune and inflammatory mechanisms

The host cellular response in terms of inflammation and immunity is also likely to be an important mediator of disease progression and pathogenesis (figure 3). Inflammation was first identified as a component of the neuropathology of Parkinson's disease in the 1980s, when microglial activation[68] and elevated inflammatory cytokines[69] were described in the post-mortem brains of patients with Parkinson's disease.

Inflammation is well described not only in the CNS, but also in the blood, with low-grade elevation of inflammatory cytokines,[70] which has been linked to more rapid disease progression.[71] Monocytes are shifted to a more inflammatory (classical) phenotype with increased expression of activation markers and proteins associated with cell migration.[72] The neutrophil to lymphocyte ratio is increased,[73] and changes in T lymphocytes include a bias towards pro-inflammatory Th1 and Th17 cell subsets[74] and a reduction in CD8 immunosenescent cells.[75] It is broadly hypothesised that infiltrating immune cells traffic to sites of neuronal damage and, together with local activated microglia, contribute to a chronic neuroinflammatory state; however, immune activation has multifaceted roles and might confer benefit, particularly in the early stages of neurodegeneration, through promoting clearance of abnormal protein aggregates. Dysfunction of immune-mediated clearance mechanisms might later contribute to aggregate accumulation, although how this protective to toxic balance shifts over time is yet to be fully resolved.[76]

The question of whether immune activation represents a primary determinant of disease progression or a secondary phenomenon has been a matter of debate for some years, but evidence from genetic and epidemiological studies provides strong support for a primary contribution to disease. The link between Parkinson's disease risk and common variation in the HLA region is now well established.[22] Monogenic causes of Parkinson's disease are also linked to the immune system: *LRRK2* is highly expressed in immune cells.[77] Use of immunosuppressants and corticosteroids was associated with substantially reduced Parkinson's disease risk in a population-based case-control study of nearly 50 000 incident Parkinson's disease cases and controls.[78]

With a background of an immunogenetic predisposition to Parkinson's disease development, several factors might drive detrimental immunological and inflammatory responses. Aberrant forms of α-synuclein can trigger an innate immune response via binding to Toll-like receptors on microglial cells and peripheral monocytes,[79] as well as inducing a specific adaptive T-cell response.[80] Furthermore, α-synuclein-specific T-cell responses in Parkinson's disease were linked to genetic risk alleles at the HLA locus, and α-synuclein peptides were shown to bind to these HLA risk variant molecules in vitro, revealing a potential mechanism for the HLA genetic association with Parkinson's disease risk.[80] Mitochondrial dysfunction in Parkinson's disease might also act as a driver of immune activation. Specifically, mitochondrial antigens are presented via MHC class I molecules to CD8 T cells, a process regulated by *PRKN* and *PINK1*.[30] Intestinal infection in *PINK1* knockout mice promotes the generation of mitochondrial-specific CD8 T cells, which traffic into the brain and are toxic to dopaminergic neurons;[81] however, whether mitochondrial autoimmunity has a role in the pathogenesis of idiopathic forms of Parkinson's disease remains to be established.

Putative environmental triggers of inflammation include infectious agents entering via the gut and nasal routes.[82] Furthermore, recent evidence indicates that α-synuclein might have an integral role in mediating the innate inflammatory response to infections, and expression of α-synuclein in enteric neurons is induced by intraperitoneal bacterial toxins in mice.[83] Changes in

the gut microbiome have also been described with a shift towards over-representation of pro-inflammatory species in Parkinson's disease, producing increased amounts of endotoxins and fewer anti-inflammatory short-chain fatty acids.[84] Gut inflammation might be linked to brain pathology via multiple routes: increased intestinal permeability and leakage of inflammatory mediators into the bloodstream and through the blood–brain barrier, promotion of α-synuclein aggregation in enteric neurons with spread of pathology via the vagus nerve, or initiation of an α-synuclein-specific T-cell response in the gut with trafficking of these T cells to sites of α-synuclein pathology in the brain, or a combination of these.[85]

Overall, there is abundant evidence for an immune component in Parkinson's disease that is closely linked to genetic predisposition to the disease, and to key elements of disease pathogenesis, including α-synuclein aggregation and mitochondrial dysfunction. Immune changes have been described from the earliest stages of the disease, including microglial activation and peripheral immune alterations in individuals with REM sleep behaviour disorder who are at high risk of developing Parkinson's disease.[86] T-cell and antibody responses might be dynamic and most prominent in early stage disease;[87,88] however, evidence regarding how the immune component of Parkinson's disease evolves over time, and whether the balance of detrimental versus neuroprotective actions varies as a function of disease stage, is not available. Determining longitudinal immune changes with greater clarity is essential for planning the optimal timing of clinical trials directed at this highly tractable pathway.

Progression

At a clinicopathological level, the onset of Parkinson's disease probably relates to the loss of a threshold proportion of substantia nigra neurons and the motor effects of loss of dopaminergic innervation of the basal ganglia. Although disease prevention trials have been proposed for people with an increased risk of development of Parkinson's disease (most notably patients with REM sleep behaviour disorder), almost all disease-modifying trials to date have been based on randomisation of a putative therapy to people with early motor disease, with a primary aim of preventing motor progression. Factors determining rapid progression in Parkinson's disease might be particularly important in guiding us to new approaches to disease modification. Conversely, slow progressors might have protective or compensatory mechanisms that could represent targets for disease modification. Currently, important factors implicated in determining the rate of progression include advanced age, impaired lysosomal function, and a pro-inflammatory state. Candidate gene studies have shown that patients with Parkinson's disease carrying single pathogenic mutations in *GBA1* have a more rapid motor and cognitive progression than patients without *GBA1* mutations.[89,90] Genome-wide progression studies indicate that *APOE* status, related amyloid processing, and synaptic dysfunction might be important determinants of survival and the development of dementia in Parkinson's disease.[91–93] Vascular and Alzheimer's copathology are probably important determinants of progression in Parkinson's disease associated with dementia (multiple protein aggregates can be present in late stage Parkinson's disease associated with dementia) and might be targets for disease-modifying therapy.[94]

Pathogenesis-driven biomarkers

Several biomarkers have been used to indicate the extent of neuronal loss and dysfunction, acting as surrogate markers for Parkinson's disease severity and progression. These biomarkers include functional imaging measures of presynaptic nigrostriatal nerve terminals; MRI measures of disruption of nigrosomes, atrophy, and iron deposition;[95] and blood neurofilament light, which is a non-specific marker of neuronal damage.[94] Progress in our understanding of the pathogenesis of Parkinson's disease has led to the development of biomarkers that directly measure certain aspects of pathogenesis (table). Given the probable heterogeneity of Parkinson's disease, these biomarkers might also allow patient stratification according to the predominant underlying mechanism (eg, mitochondrial, lysosomal, or immune), enabling targeted patient selection for future clinical trials.

α-Synuclein and phosphorylated α-synuclein can be directly measured in blood, although there is an overlap between patients with Parkinson's disease and healthy controls.[94] Studies of the enhanced aggregation of α-synuclein and studies of the propagation of Parkinson's pathology have led to the development of seeding assays (real time quaking-induced conversion) based on the ability of patient samples, including cerebrospinal fluid, skin biopsies, saliva, and olfactory biopsies, to trigger α-synuclein aggregation in vitro.[96–99] These assays show promise in improving the early diagnosis of Parkinson's disease, and in distinguishing Parkinson's disease, multiple system atrophy, and unaffected controls. These assays have been incorporated into proposed biological staging systems for synuclein disorders encompassing individuals both who are at risk and who are clinically affected by motor symptoms, with a common underlying disease process, analogously to the biological definition of Alzheier's disease.[100,101] Serum markers of mitochondrial disease have not shown group differences between Parkinson's disease and controls; however, in-depth analysis of fibroblasts from patients with Parkinson's disease suggests that defining mitochondrial subgroups of these patients might be possible, highlighting the heterogeneity of the pathogenesis of the disease.[102,103] Magnetic resonance spectroscopy is a promising approach to assessing mitochondrial dysfunction. A reduction in ATP can be

defined in some patients with Parkinson's disease as compared with controls, and has been incorporated as a secondary outcome measure in trials.[104,105]

Specific aspects of lysosomal function have been measured in patients with Parkinson's disease, directed by primary genetic cause. BMPs are associated with late endosomal and lysosomal membranes, and changes in levels of urinary BMPs have been identified in animal models of LRRK2 dysfunction. *LRRK2* Gly2019Ser mutation carriers have elevated levels of urinary BMPs, presumably reflecting changes in endosomal-lysosomal structure.[106,107] Direct assays of LRRK2 kinase activity can be done through cellular assays of Rab proteins, which might represent the main physiological substrate for LRRK2 kinases.[108,109] The biochemistry of Gcase can be measured through assays of GCase activity, GCase protein concentrations, and concentrations of the glucosylceramide substrate together with other glycosphingolipids affected by changes in GCase. Brain inflammation in the living patient with Parkinson's disease has been shown in PET neuroimaging studies using ligands for the mitochondrial 18-kDa translocator protein, which is upregulated in activated glial cells.[110] Cytokine concentrations in blood and cerebrospinal fluid are variable across studies, but a meta-analysis provides evidence for elevated TGFB1, IL6R, and IL1B.[111]

Pathogenesis-driven drug trials

To date, no disease-modifying, neuroprotective treatments for Parkinson's disease have been shown to be effective in phase 3 clinical trials; however, as understanding of the pathogenesis of Parkinson's disease rapidly expands, multiple promising drug targets that might have potential as disease-modifying agents and warrant further consideration in early-phase trials have been identified (table).[2] The concept of total *SNCA* gene expression as a primary driver of disease has led to the development of antisense oligonucleotide therapy approaches, which aims to suppress the production of α-synuclein in the CNS. Antiaggregation therapy has shown efficacy in transgenic mouse models, and at least two agents (anle138b [MODAG, Wendelsheim, Germany] and NPT200-11 [Neuropore Therapies, San Diego, CA, USA]) have been trialled in phase 1 studies.[2,112] Several putative therapies targeting mitochondrial function and oxidative stress, including inosine and ubidecarenone (also known as coenzyme Q10), did not meet their primary endpoints. Another phase 2 trial of ursodeoxycholic acid is ongoing.[105] The emerging data on cell-to-cell spread of α-synuclein pathology have led to treatment trials based on active and passive immunisation against α-synuclein. Two recent trials using peripheral administration of monoclonal antibodies against α-synuclein did not meet their prespecified primary endpoints, despite promising preclinical studies.[113,114] Trials of anti-inflammatory agents targeting microglial activation, including minocycline and pioglitazone, have not shown clinical benefit.[115,116] A phase 1 trial has shown that sargramostim (a granulocyte-macrophage colony-stimulating factor) boosts T regulatory cell numbers in patients with Parkinson's disease with acceptable safety data.[117] A phase 2 trial of a peripherally acting immunosuppressant drug, azathioprine, is currently underway.[118]

Other trials have been designed to target specific genes, or genetic variants, or both.[2] Whether these agents will be effective in all patients with Parkinson's disease, or only in the subset carrying a specific mutation, is unknown. Most of these studies have included patients with both sporadic and monogenic Parkinson's disease. Planned studies directed towards *GBA1* include modulators of GCase, including ambroxol and LTI-291 (Lysosomal Therapeutics, Cambridge, MA, USA), and a *GBA1* gene replacement therapy. A trial of substrate reduction with venglustat was unsuccessful in slowing the 1-year progression of Parkinson's disease in a phase 2 study. Finally, *LRRK2* has emerged as a primary target for therapeutic trials with ongoing trials of an antisense oligonucleotide, which will suppress the expression of *LRRK2*, and of oral LRRK2 kinase inhibitors.

The failure of trials to show efficacy in modifying the course of Parkinson's disease so far might relate to an incomplete understanding of the pathogenesis of the disease, intervention too late in the disease course, incomplete mechanism-based patient selection, a study duration that is too short, or use of clinical outcome measures that do not adequately capture disease progression.[2] Post-hoc analyses based on emerging genetic and pathogenesis markers might provide more information on targeting clinical trials for successful outcomes. The incremental increase in our understanding of Parkinson's disease pathogenesis will improve our ability to select drug targets, improve patient stratification, and develop markers of target engagement.

Conclusions

Advances in the understanding of the pathogenesis of Parkinson's disease, driven by neurogenetics, have provided insights into the initiation and progression of the disease. Parkinson's disease relates to the formation of abnormal α-synuclein aggregates both in the periphery and the brain, as well as the spread of this pathology through the brain. This pathology is accompanied by immune activation, neuroinflammation, mitochondrial dysfunction, and changes in lysosomal and endosomal function. Multiple lines of evidence support these disparate pathological processes in Parkinson's disease, as well as evidence of overlap and convergence. Examples of this overlap are *LRRK2* and *PRKN*, which seem to be important in both endo-lysosomal and mitochondrial function, and might also have a role in regulating immune responses. The importance of these processes is difficult to establish in idiopathic Parkinson's disease; however, in mendelian forms of Parkinson's disease, the primary genetic cause is known, and consideration of the

different pathological processes in these genetic cases provides some insight into the mechanistic basis of clinical disease heterogeneity. For example, patients with *PRKN* mutations and primary mitochondrial dysfunction have a restricted pattern of cell loss that is largely confined to the dopaminergic nigrostriatal system, without the widespread pathology and non-motor features seen in typical sporadic Parkinson's disease. Conversely, patients with *SNCA* or *GBA* mutations and prominent α-synuclein Lewy body pathology have early non-motor features, including autonomic dysfunction and dementia, presumably reflecting widespread pathology throughout the periphery and brain. Whether these mechanistic and pathological links to clinical heterogeneity seen in genetic forms of Parkinson's disease are also applicable to sporadic, polygenic Parkinson's disease is unclear, and determining these relationships is a major challenge in current research.

Despite recent advances, many controversies remain unresolved. These controversies include the contribution of peripheral α-synuclein pathology to disease initiation; the mechanism by which pathology spreads from the periphery to the brain, and within the brain; the size and conformation of the α-synuclein aggregates that are most neurotoxic; the balance of neuroprotective versus neurotoxic effects of immune activation and how it varies through the disease course; the role of infectious and microbial agents, including changes in the gut microbiome, in driving the immune component of the disease; and the role of copathologies such as tau, amyloid β aggregation, and vasculopathy in contributing to disease progression. These issues are key research priorities that need to be addressed through a combination of preclinical research and longitudinal clinicogenetic studies to inform future therapeutic strategies. Despite the complexity and ongoing controversies surrounding the pathogenesis of Parkinson's disease, we should move from the dopamine replacement era to the era of disease modification, and we hope that the major scientific advances outlined here will lead to new treatments for patients with Parkinson's disease.

Contributors

HRM did the literature search, prepared the first and final drafts, contributed to the Introduction, Clinical aspects of Parkinson's disease, Genetics, Progression, Pathogenesis-driven biomarkers, Pathogenesis-driven drug trials, and Conclusions sections, and prepared the table. MGS prepared the Neuropathology section and figure 1. CMS prepared the Molecular mechanisms contributing to Parkinson's disease section and prepared figure 2. CHW-G wrote the Immune and inflammatory mechanisms, Pathogenesis-driven biomarkers, Pathogenesis-driven drug trials, and Conclusions sections. All authors contributed to writing the final version of the systematic review and approved the final version for publication.

Declaration of interests

HRM is employed by University College London, and in the past 36 months reports paid consultancy from Roche, Aprinoia, and Amylyx; lecture fees and honoraria from the British Medical Journal, Kyowa Kirin, and the Movement Disorder Society; research grants from CBD Solutions, Drake Foundation, Parkinson's UK, Cure Parkinson's Trust, PSP Association, Medical Research Council, and the Michael J Fox Foundation (MJFF); and is a coapplicant on a patent application related to C9ORF72—method for diagnosing a neurodegenerative disease (PCT/GB2012/052140).
MGS is a William Scholl Professor supported by the Scholl Foundation endowment to Cambridge University, is supported by the Wellcome Trust, is supported by the NIHR Cambridge Biomedical Research Centre Dementia (NIHR203312) and Neurodegeneration Theme (146281), and in the past 12 months has been paid lecture fees or honoraria, or both, from the Movement Disorder Society and Institute San Raffaele; is on the Scientific Advisory Board of the Tau Consortium, Qatar Biomedical Research Institute, Fondazione Don Gnocchi, Eurac, and the European Brain Research Institute; is a consultant for Astex; is in scientific collaboration with Eli Lilly and Teva; and reports research grants from Parkinson's UK, MJFF, Biotechnology and Biological Sciences Research Council, the Scholl Foundation, Fondation de la Recherche Alzheimer, Alzheimer's Research UK, Medical Research Council, Wellcome Trust, and the Bill & Melinda Gates foundation. CMS is employed by South Eastern Sydney Local Health District, Neuroscience Research Australia, and University of New South Wales; is a National Health and Medical Research Council (NHMRC) Practitioner Fellow (APP1136800); and in the past 12 months, has received research funding from the NHMRC, the Medical Research Futures Fund, MJFF, and Shake It Up Australia; and has been paid lecture fees and honoraria from the Movement Disorder Society and the Taiwan Movement Disorder Society.
CHW-G is employed by the University of Cambridge, and in the past 12 months has received research funding from the Medical Research Council (MR/W029235/1), the National Institutes of Health Research (NIHR) Cambridge Biomedical Research Centre (NIHR203312), Parkinson's UK, Cure Parkinson's, the Evelyn Trust, and the Cambridge Centre for Parkinson-Plus; consultancy fees from Evidera; and speaker fees from GSK and the World Parkinson Congress.

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References

1 Ben-Shlomo Y, Darweesh S, Llibre-Guerra J, et al. The epidemiology of Parkinson's disease. *Lancet* 2024; **403:** 293–3-4.
2 Vijiaratnam N, Simuni T, Bandmann O, Morris HR, Foltynie T. Progress towards therapies for disease modification in Parkinson's disease. *Lancet Neurol* 2021; **20:** 559–72.
3 Lewy FH. Paralysis agitans. I. Pathologische anatomie. In: Lewandowsky M ed. Handbuch der Neurologie. Berlin: Springer Verlag, 1912: 920–33.
4 Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J Neurol Neurosurg Psychiatry* 1988; **51:** 745–52.
5 Geddes JF, Hughes AJ, Lees AJ, Daniel SE. Pathological overlap in cases of parkinsonism associated with neurofibrillary tangles. A study of recent cases of postencephalitic parkinsonism and comparison with progressive supranuclear palsy and Guamanian parkinsonism-dementia complex. *Brain* 1993; **116:** 281–302.
6 Kalia LV, Lang AE, Hazrati LN, et al. Clinical correlations with Lewy body pathology in LRRK2-related Parkinson disease. *JAMA Neurol* 2015; **72:** 100–05.
7 Doherty KM, Silveira-Moriyama L, Parkkinen L, et al. Parkin disease: a clinicopathologic entity? *JAMA Neurol* 2013; **70:** 571–79.
8 Wang J-L, Xiao B, Cui X-X, et al. Analysis of SCA2 and SCA3/MJD repeats in Parkinson's disease in mainland China: genetic, clinical, and positron emission tomography findings. *Mov Disord* 2009; **24:** 2007–11.
9 Caparros-Lefebvre D, Sergeant N, Lees A, et al. Guadeloupean parkinsonism: a cluster of progressive supranuclear palsy-like tauopathy. *Brain* 2002; **125:** 801–11.
10 Steele JC, McGeer PL. The ALS/PDC syndrome of Guam and the cycad hypothesis. *Neurology* 2008; **70:** 1984–90.
11 Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord* 2015; **30:** 1591–601.

12 Blauwendraat C, Nalls MA, Singleton AB. The genetic architecture of Parkinson's disease. *Lancet Neurol* 2020; **19:** 170–78.

13 Gegg ME, Menozzi E, Schapira AHV. Glucocerebrosidase-associated Parkinson disease: pathogenic mechanisms and potential drug treatments. *Neurobiol Dis* 2022; **166:** 105663.

14 Kluss JH, Mamais A, Cookson MR. LRRK2 links genetic and sporadic Parkinson's disease. *Biochem Soc Trans* 2019; **47:** 651–61.

15 Xie CL, Pan JL, Wang WW, et al. The association between the LRRK2 G2385R variant and the risk of Parkinson's disease: a meta-analysis based on 23 case-control studies. *Neurol Sci* 2014; **35:** 1495–504.

16 Goedert M, Jakes R, Spillantini MG. The synucleinopathies: twenty years on. *J Parkinsons Dis* 2017; **7** (suppl 1)**:** S51–69.

17 Goedert M, Spillantini MG, Del Tredici K, Braak H. 100 years of Lewy pathology. *Nat Rev Neurol* 2013; **9:** 13–24.

18 Singleton A, Hardy J. A generalizable hypothesis for the genetic architecture of disease: pleomorphic risk loci. *Hum Mol Genet* 2011; **20:** R158–62.

19 Rizig M, Bandres-Ciga S, Makarious MB, et al. Genome-wide association identifies novel etiological insights associated with Parkinson's disease in African and African admixed populations. *medRxiv* 2023; published onlilne May 5. https://www.medrxiv.org/content/10.1101/2023.05.05.23289529v1 (preprint).

20 Soutar MPM, Melandri D, O'Callaghan B, et al. Regulation of mitophagy by the NSL complex underlies genetic risk for Parkinson's disease at 16q11.2 and MAPT H1 loci. *Brain* 2022; **145:** 4349–67.

21 Holmans P, Moskvina V, Jones L, et al. A pathway-based analysis provides additional support for an immune-related genetic susceptibility to Parkinson's disease. *Hum Mol Genet* 2013; **23:** 562.

22 Nalls MA, Blauwendraat C, Vallerga CL, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet Neurol* 2019; **18:** 1091–102.

23 Duffy PE, Tennyson VM. Phase and electron microscopic observations of Lewy bodies and melanin granules in the substantia nigra and locus caeruleus in Parkinson's disease. *J Neuropathol Exp Neurol* 1965; **24:** 398–414.

24 Guo Y, Sun Y, Song Z, et al. Genetic analysis and literature review of *SNCA* variants in Parkinson's disease. *Front Aging Neurosci* 2021; **13:** 648151.

25 Yang Y, Shi Y, Schweighauser M, et al. Cryo-EM structures of α-synuclein filaments from Parkinson's disease and dementia with Lewy bodies. *bioRxiv* 2022; published online July 12. https://doi.org/10.1101/2022.07.12.499706 (preprint).

26 Spillantini MG, Schmidt ML, Lee VMY, Trojanowski JQ, Jakes R, Goedert M. α-Synuclein in Lewy bodies. *Nature* 1997; **388:** 839–40.

27 Tozzi A, Sciaccaluga M, Loffredo V, et al. Dopamine-dependent early synaptic and motor dysfunctions induced by α-synuclein in the nigrostriatal circuit. *Brain* 2021; **144:** 3477–91.

28 Calo L, Wegrzynowicz M, Santivañez-Perez J, Grazia Spillantini M. Synaptic failure and α-synuclein. *Mov Disord* 2016; **31:** 169–77.

29 Schweighauser M, Shi Y, Tarutani A, et al. Structures of α-synuclein filaments from multiple system atrophy. *Nature* 2020; **585:** 464–69.

30 Lobanova E, Whiten D, Ruggeri FS, et al. Imaging protein aggregates in the serum and cerebrospinal fluid in Parkinson's disease. *Brain* 2022; **145:** 632–43.

31 Braak H, Tredici KD, Rüb U, Vos RAI de, Steur ENHJ, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 2003; **24:** 197–211.

32 Alam P, Bousset L, Melki R, Otzen DE. α-Synuclein oligomers and fibrils: a spectrum of species, a spectrum of toxicities. *J Neurochem* 2019; **150:** 522–34.

33 Schrag A, Horsfall L, Walters K, Noyce A, Petersen I. Prediagnostic presentations of Parkinson's disease in primary care: a case-control study. *Lancet Neurol* 2015; **14:** 57–64.

34 Dickson DW, Braak H, Duda JE, et al. Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. *Lancet Neurol* 2009; **8:** 1150–57.

35 Horsager J, Andersen KB, Knudsen K, et al. Brain-first versus body-first Parkinson's disease: a multimodal imaging case-control study. *Brain* 2020; **143:** 3077–88.

36 Beach TG, Adler CH, Lue L, et al. Unified staging system for Lewy body disorders: correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. *Acta Neuropathol* 2009; **117:** 613–34.

37 Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med* 2008; **14:** 504–06.

38 Li JY, Englund E, Holton JL, et al. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat Med* 2008; **14:** 501–03.

39 Luk KC, Kehm V, Carroll J, et al. Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science* 2012; **338:** 949–53.

40 Recasens A, Ulusoy A, Kahle PJ, Di Monte DA, Dehay B. In vivo models of alpha-synuclein transmission and propagation. *Cell Tissue Res* 2018; **373:** 183–93.

41 Arotcarena ML, Dovero S, Prigent A, et al. Bidirectional gut-to-brain and brain-to-gut propagation of synucleinopathy in non-human primates. *Brain* 2020; **143:** 1462–75.

42 Chu Y, Muller S, Tavares A, et al. Intrastriatal alpha-synuclein fibrils in monkeys: spreading, imaging and neuropathological changes. *Brain* 2019; **142:** 3565–79.

43 Ross GW, Abbott RD, Petrovitch H, Tanner CM, White LR. Pre-motor features of Parkinson's disease: the Honolulu-Asia Aging Study experience. *Parkinsonism Relat Disord* 2012; **18** (suppl 1)**:** S199–202.

44 Bohnen NI, Hu MTM. Sleep disturbance as potential risk and progression factor for Parkinson's disease. *J Parkinsons Dis* 2019; **9:** 603–14.

45 Nishioka K, Imai Y, Yoshino H, Li Y, Funayama M, Hattori N. Clinical manifestations and molecular backgrounds of Parkinson's Disease regarding genes identified from familial and population studies. *Front Neurol* 2022; **13:** 764917.

46 Vrijsen S, Vrancx C, Del Vecchio M, et al. Inter-organellar communication in Parkinson's and Alzheimer's disease: looking beyond endoplasmic reticulum-mitochondria contact sites. *Front Neurosci* 2022; **16:** 900338.

47 Sironi L, Restelli LM, Tolnay M, Neutzner A, Frank S. Dysregulated interorganellar crosstalk of mitochondria in the pathogenesis of Parkinson's disease. *Cells* 2020; **9:** 233.

48 Grünewald A, Kumar KR, Sue CM. New insights into the complex role of mitochondria in Parkinson's disease. *Prog Neurobiol* 2019; **177:** 73–93.

49 Nicoletti V, Palermo G, Del Prete E, Mancuso M, Ceravolo R. Understanding the multiple role of mitochondria in Parkinson's disease and related disorders: lesson from genetics and protein-interaction network. *Front Cell Dev Biol* 2021; **9:** 636506.

50 Langston JW. The MPTP story. *J Parkinsons Dis* 2017; **7:** S11–19.

51 Mani S, Sevanan M, Krishnamoorthy A, Sekar S. A systematic review of molecular approaches that link mitochondrial dysfunction and neuroinflammation in Parkinson's disease. *Neurol Sci* 2021; **42:** 4459–69.

52 Li W, Fu Y, Halliday GM, Sue CM. *PARK* genes link mitochondrial dysfunction and alpha-synuclein pathology in sporadic Parkinson's disease. *Front Cell Dev Biol* 2021; **9:** 612476.

53 Terešak P, Lapao A, Subic N, Boya P, Elazar Z, Simonsen A. Regulation of PRKN-independent mitophagy. *Autophagy* 2022; **18:** 24–39.

54 Eldeeb MA, Thomas RA, Ragheb MA, Fallahi A, Fon EA. Mitochondrial quality control in health and in Parkinson's disease. *Physiol Rev* 2022; **102:** 1721–55.

55 Wang XL, Feng ST, Wang YT, et al. Mitophagy, a form of selective autophagy, plays an essential role in mitochondrial dynamics of Parkinson's disease. *Cell Mol Neurobiol* 2022; **42:** 1321–39.

56 Singh F, Ganley IG. Parkinson's disease and mitophagy: an emerging role for LRRK2. *Biochem Soc Trans* 2021; **49:** 551–62.

57 Haque ME, Akther M, Azam S, et al. Targeting α-synuclein aggregation and its role in mitochondrial dysfunction in Parkinson's disease. *Br J Pharmacol* 2022; **179:** 23–45.

58 Burtscher J, Syed MMK, Keller MA, Lashuel HA, Millet GP. Fatal attraction - the role of hypoxia when alpha-synuclein gets intimate with mitochondria. *Neurobiol Aging* 2021; **107:** 128–41.

59 Picca A, Guerra F, Calvani R, et al. Mitochondrial dysfunction, protein misfolding and neuroinflammation in Parkinson's disease: roads to biomarker discovery. *Biomolecules* 2021; **11:** 1508.

60 Horowitz M, Braunstein H, Zimran A, Revel-Vilk S, Goker-Alpan O. Lysosomal functions and dysfunctions: molecular and cellular mechanisms underlying Gaucher disease and its association with Parkinson disease. *Adv Drug Deliv Rev* 2022; **187:** 114402.

61 Navarro-Romero A, Montpeyó M, Martinez-Vicente M. The emerging role of the lysosome in Parkinson's disease. *Cells* 2020; **9:** 2399.

62 Brooker SM, Krainc D. Glucocerebrosidase dysfunction in neurodegenerative disease. *Essays Biochem* 2021; **65:** 873–83.

63 Bo RX, Li YY, Zhou TT, Chen NH, Yuan YH. The neuroinflammatory role of glucocerebrosidase in Parkinson's disease. *Neuropharmacology* 2022; **207:** 108964.

64 Smith L, Schapira AHV. *GBA* variants and Parkinson disease: mechanisms and treatments. *Cells* 2022; **11:** 1261.

65 Giladi N, Alcalay RN, Cutter G, et al. Safety and efficacy of venglustat in GBA1-associated Parkinson's disease: an international, multicentre, double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet Neurol* 2023; **22:** 661–71.

66 Erb ML, Moore DJ. LRRK2 and the endolysosomal system in Parkinson's disease. *J Parkinsons Dis* 2020; **10:** 1271–91.

67 Di Maio R, Hoffman EK, Rocha EM, et al. LRRK2 activation in idiopathic Parkinson's disease. *Sci Transl Med* 2018; **10:** eaar5429.

68 McGeer PL, Itagaki S, Boyes BE, McGeer EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology* 1988; **38:** 1285–91.

69 Mogi M, Harada M, Kondo T, et al. Interleukin-1 β, interleukin-6, epidermal growth factor and transforming growth factor-α are elevated in the brain from parkinsonian patients. *Neurosci Lett* 1994; **180:** 147–50.

70 Qin XY, Zhang SP, Cao C, Loh YP, Cheng Y. Aberrations in peripheral inflammatory cytokine levels in Parkinson disease: a systematic review and meta-analysis. *JAMA Neurol* 2016; **73:** 1316–24.

71 Williams-Gray CH, Wijeyekoon R, Yarnall AJ, et al. Serum immune markers and disease progression in an incident Parkinson's disease cohort (ICICLE-PD). *Mov Disord* 2016; **31:** 995–1003.

72 Nissen SK, Farmen K, Carstensen M, et al. Changes in CD163+, CD11b+, and CCR2+ peripheral monocytes relate to Parkinson's disease and cognition. *medRxiv* 2021; published online Dec 15. https://doi.org/10.1101/2021.03.15.21253572 (preprint).

73 Muñoz-Delgado L, Macías-García D, Jesús S, et al. Peripheral immune profile and neutrophil-to-lymphocyte ratio in Parkinson's disease. *Mov Disord* 2021; **36:** 2426–30.

74 Contaldi E, Magistrelli L, Comi C. T lymphocytes in Parkinson's disease. *J Parkinsons Dis* 2022; **12:** S65–74.

75 Kouli A, Williams-Gray CH. Age-related adaptive immune changes in Parkinson's disease. *J Parkinsons Dis* 2022; **12:** S93–104.

76 Weiss F, Labrador-Garrido A, Dzamko N, Halliday G. Immune responses in the Parkinson's disease brain. *Neurobiol Dis* 2022; **168:** 105700.

77 Magistrelli L, Contaldi E, Vignaroli F, et al. Immune response modifications in the genetic forms of Parkinson's disease: what do we know? *Int J Mol Sci* 2022; **23:** 3476.

78 Racette BA, Gross A, Vouri SM, Camacho-Soto A, Willis AW, Searles Nielsen S. Immunosuppressants and risk of Parkinson disease. *Ann Clin Transl Neurol* 2018; **5:** 870–75.

79 Kouli A, Horne CB, Williams-Gray CH. Toll-like receptors and their therapeutic potential in Parkinson's disease and α-synucleinopathies. *Brain Behav Immun* 2019; **81:** 41–51.

80 Sulzer D, Alcalay RN, Garretti F, et al. T cells from patients with Parkinson's disease recognize α-synuclein peptides. *Nature* 2017; **546:** 656–61.

81 Matheoud D, Sugiura A, Bellemare-Pelletier A, et al. Parkinson's disease-related proteins PINK1 and parkin repress mitochondrial antigen presentation. *Cell* 2016; **166:** 314–27.

82 Matheoud D, Cannon T, Voisin A, et al. Intestinal infection triggers Parkinson's disease-like symptoms in Pink1$^{-/-}$ mice. *Nature* 2019; **571:** 565–69.

83 Alam MM, Yang D, Li XQ, et al. Alpha synuclein, the culprit in Parkinson disease, is required for normal immune function. *Cell Rep* 2022; **38:** 110090.

84 Romano S, Savva GM, Bedarf JR, Charles IG, Hildebrand F, Narbad A. Meta-analysis of the Parkinson's disease gut microbiome suggests alterations linked to intestinal inflammation. *NPJ Parkinsons Dis* 2021; **7:** 27.

85 Houser MC, Tansey MG. The gut-brain axis: is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? *NPJ Parkinsons Dis* 2017; **3:** 3.

86 Farmen K, Nissen SK, Stokholm MG, et al. Monocyte markers correlate with immune and neuronal brain changes in REM sleep behavior disorder. *Proc Natl Acad Sci USA* 2021; **118:** e2020858118.

87 Scott KM, Kouli A, Yeoh SL, Clatworthy MR, Williams-Gray CH. A systematic review and meta-analysis of alpha synuclein auto-antibodies in Parkinson's disease. *Front Neurol* 2018; **9:** 815.

88 Lindestam Arlehamn CS, Dhanwani R, Pham J, et al. α-Synuclein-specific T cell reactivity is associated with preclinical and early Parkinson's disease. *Nat Commun* 2020; **11:** 1875.

89 Liu G, Boot B, Locascio JJ, et al. Specifically neuropathic Gaucher's mutations accelerate cognitive decline in Parkinson's. *Ann Neurol* 2016; **80:** 674–85.

90 Stoker TB, Camacho M, Winder-Rhodes S, et al. Impact of *GBA1* variants on long-term clinical progression and mortality in incident Parkinson's disease. *J Neurol Neurosurg Psychiatry* 2020; **91:** 695–702.

91 Tan MMX, Lawton MA, Jabbari E, et al. Genome-wide association studies of cognitive and motor progression in Parkinson's disease. *Mov Disord* 2021; **36:** 424–33.

92 Liu G, Peng J, Liao Z, et al. Genome-wide survival study identifies a novel synaptic locus and polygenic score for cognitive progression in Parkinson's disease. *Nat Genet* 2021; **53:** 787–93.

93 Real R, Martinez-Carrasco A, Reynolds RH, et al. Association between the LRP1B and APOE loci and the development of Parkinson's disease dementia. *Brain* 2023; **146:** 1873–87.

94 Morris HR. Blood based biomarkers for movement disorders. *Acta Neurol Scand* 2022; **146:** 353–61.

95 Algarni MA, Stoessl AJ. The role of biomarkers and imaging in Parkinson's disease. *Expert Rev Neurother* 2016; **16:** 187–203.

96 Shahnawaz M, Mukherjee A, Pritzkow S, et al. Discriminating α-synuclein strains in Parkinson's disease and multiple system atrophy. *Nature* 2020; **578:** 273–77.

97 Stefani A, Iranzo A, Holzknecht E, et al. Alpha-synuclein seeds in olfactory mucosa of patients with isolated REM sleep behaviour disorder. *Brain* 2021; **144:** 1118–26.

98 Siderowf A, Concha-Marambio L, Lafontant D-E, 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:** 407–17.

99 Vivacqua G, Mason M, Bartolo MID, et al. Salivary α-synuclein RT-QuIC correlates with disease severity in de novo Parkinson's disease. *Mov Disord* 2023; **38:** 153–55.

100 Höglinger GU, Adler CH, Berg D, et al. Towards a biological definition of Parkinson's disease. *Preprints* 2023; published online April 5. https://www.preprints.org/manuscript/202304.0108/v1 (preprint).

101 Chahine LM, Merchant K, Siderowf A, et al. Proposal for a biologic staging system of Parkinson's disease. *J Parkinsons Dis* 2023; **13:** 297–309.

102 Carling PJ, Mortiboys H, Green C, et al. Deep phenotyping of peripheral tissue facilitates mechanistic disease stratification in sporadic Parkinson's disease. *Prog Neurobiol* 2020; **187:** 101772.

103 Davis RL, Wong SL, Carling PJ, Payne T, Sue CM, Bandmann O. Serum FGF-21, GDF-15, and blood mtDNA copy number are not biomarkers of Parkinson disease. *Neurol Clin Pract* 2020; **10:** 40–46.

104 Hattingen E, Magerkurth J, Pilatus U, et al. Phosphorus and proton magnetic resonance spectroscopy demonstrates mitochondrial dysfunction in early and advanced Parkinson's disease. *Brain* 2009; **132:** 3285–97.

105 Payne T, Sassani M, Buckley E, et al. Ursodeoxycholic acid as a novel disease-modifying treatment for Parkinson's disease: protocol for a two-centre, randomised, double-blind, placebo-controlled trial, the 'UP' study. *BMJ Open* 2020; **10:** e038911.

106 Merchant K, Simuni T, Fedler J, et al. LRRK2 and GBA1 variant carriers have higher urinary bis(monacylglycerol) phosphate concentrations in PPMI cohorts. *NPJ Parkinsons Dis* 2023; **9:** 30.

107 Alcalay RN, Hsieh F, Tengstrand E, et al. Higher urine bis(monoacylglycerol)phosphate levels in LRRK2 G2019S mutation carriers: implications for therapeutic development. *Mov Disord* 2020; **35:** 134–41.

108 Fan Y, Nirujogi RS, Garrido A, et al. R1441G but not G2019S mutation enhances LRRK2 mediated Rab10 phosphorylation in human peripheral blood neutrophils. *Acta Neuropathol* 2021; **142:** 475–94.

109 Fan Y, Tonelli F, Padmanabhan S, et al. Human peripheral blood neutrophil isolation for interrogating the Parkinson's associated LRRK2 kinase pathway by assessing Rab10 phosphorylation. *J Vis Exp* 2020; **157:** e58956.

110 Zhang PF, Gao F. Neuroinflammation in Parkinson's disease: a meta-analysis of PET imaging studies. *J Neurol* 2022; **269:** 2304–14.

111 Chen X, Hu Y, Cao Z, Liu Q, Cheng Y. Cerebrospinal fluid inflammatory cytokine aberrations in Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis: a systematic review and meta-analysis. *Front Immunol* 2018; **9:** 2122.

112 Wegrzynowicz M, Bar-On D, Calo' L, et al. Depopulation of dense α-synuclein aggregates is associated with rescue of dopamine neuron dysfunction and death in a new Parkinson's disease model. *Acta Neuropathol* 2019; **138:** 575–95.

113 Lang AE, Siderowf AD, Macklin EA, et al. Trial of cinpanemab in early Parkinson's disease. *N Engl J Med* 2022; **387:** 408–20.

114 Pagano G, Taylor KI, Anzures-Cabrera J, et al. Trial of prasinezumab in early-stage Parkinson's disease. *N Engl J Med* 2022; **387:** 421–32.

115 Investigators NET. Pioglitazone in early Parkinson's disease: a phase 2, multicentre, double-blind, randomised trial. *Lancet Neurol* 2015; **14:** 795–803.

116 Investigators TNNP. A randomized, double-blind, futility clinical trial of creatine and minocycline in early Parkinson disease. *Neurology* 2006; **66:** 664–71.

117 Gendelman HE, Zhang Y, Santamaria P, et al. Evaluation of the safety and immunomodulatory effects of sargramostim in a randomized, double-blind phase 1 clinical Parkinson's disease trial. *NPJ Parkinsons Dis* 2017; **3:** 10.

118 Greenland JC, Cutting E, Kadyan S, Bond S, Chhabra A, Williams-Gray CH. Azathioprine immunosuppression and disease modification in Parkinson's disease (AZA-PD): a randomised double-blind placebo-controlled phase II trial protocol. *BMJ Open* 2020; **10:** e040527.