The pathogenesis of Parkinson's disease

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

Introduction: Parkinson's disease (PD) is a common neurodegenerative disease which causes major disability and an increasing global public health burden related to motor, non-motor and cognitive features (**Ref:** Lancet PD Epidemiology Review). Advances in the genetics of PD, beginning with the identification of alpha-synuclein as the first autosomal dominant gene for PD, have led to a rapid increase in our understanding of its pathogenesis. The major challenges include the 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 PD disease modifying trials are planned or in progress, directly related to these recent advances [1]. Some trials are now selecting patients with specific genetic variants and it is possible that future therapies will be targeted to disease mechanisms in a stratified medicine approach based on biomarkers, as well as genotype. This review summarises recent advances in our understanding of the pathogenesis of PD and highlights likely future developments.

Clinical aspects of Parkinson's disease : PD is a clinico-pathological syndrome in which progressive asymmetric slowness of movement (bradykinesia), rigidity, tremor and gait disturbance, is associated with neuronal loss and the formation of alpha-synuclein containing proteinaceous aggregates in neurons of the substantia nigra known as Lewy bodies (LBs), and Lewy neurites [2,3]. However, parkinsonism may have diverse pathological underlying causes including tau, polyglutamine and Alzheimer pathology, and nigral cell loss without hallmark pathological features [4–6]. Some of these causes of non-Lewy body parkinsonism have been reported in non-European populations [5,7–9]. Lewy body PD is the primary focus of this review but it is possible that a broader concept of the pathogenesis of parkinsonism is needed to describe the relevant disease mechanisms in different parts of the world.

The definition of PD in the Queen Square Brain Bank clinical diagnostic criteria primarily related to the levodopa responsive motor phenotype, directly related to cell loss in the substantia nigra, and dopaminergic denervation of the caudate and putamen [3]. Although the non-motor features in PD have been described from the earliest descriptions of the disease, clinico-pathological work over the last 20 years has focussed major interest on the involvement of other neural systems which affect the gut, autonomic nervous system, sleep, smell, anxiety and cognition. The Movement Disorders Society (MDS) clinical diagnostic criteria for PD include weighting for these non-motor features of PD, which are often helpful in arriving at a clinical diagnosis and are recognised as central determinants of impaired quality of life and morbidity for PD patients [10]. PD non-motor features may also provide important clues as to the underlying disease pathogenesis.

PD is a progressive condition and studies of disease pathogenesis encompass both the initiation of the disease and the development of progressively worsening clinical features,

with the involvement of multiple brain regions. Here we will review the pathogenesis of PD 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: PD 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 [11]. The heritability of PD/parkinsonism has been estimated from both twin studies and statistical genetic methods to lie between 22% and 40%, so the aetiology of PD is likely to have a substantial genetic and environmental component (**Lancet PD Epidemiology review**). There are three well validated autosomal dominant and three well validated autosomal recessive genes that cause PD (SNCA, LRRK2 and VPS35; and PRKN, PINK1 and DJ1), together with a series of genes that have been reported in small numbers of cases/families. In addition, common and single rare variants in the Gaucher disease (GD) causing gene *GBA1*, encoding glucocerebrosidase, are associated with PD but usually do not segregate in autosomal dominant families [12]. Numerically, LRRK2 is the most important Mendelian cause of PD with the G2019S mutation occurring most commonly in European/North African/Jewish kindreds, N1437D mutation in Chinese kindreds and I2020T in Japanese kindreds [13]. Additionally the LRRK2 G2385R variant is a risk factor for PD in the Chinese and Korean populations with an odds ratio of ~ 2.4 [14]. LRRK2 is also implicated in the development of Crohn's disease, leprosy and mycobacterial disease and there may be pleiotropic effects at the LRRK2 locus regulating susceptibility and resistance to infectious/inflammatory disease and neurodegeneration [13].

SNCA mutations are a rarer cause of autosomal dominant PD. The identification of the Ala53Thr mutation in the *SNCA* gene in an Italian-American family with autosomal dominant PD, led to the identification of alpha-synuclein protein as the hallmark component of Lewy bodies and Lewy neurites and glial cytoplasmic inclusions in multiple system atrophy (MSA) [15,16]. The identification of pathogenic *SNCA* gene multiplications showed that an increase in the level of production of SNCA mRNA and alpha-synuclein could be sufficient to cause PD [17]. Many *SNCA* mutation families have prominent cortical Lewy body formation, autonomic failure and pathology that overlaps between MSA, DLB and PD indicating shared mechanisms and clinical features across these disorders. They may also have early disease onset and rapid progression to poor outcomes indicating that SNCA related mechanisms may be a driver of disease severity.

Genome wide association studies (GWAS) have been used to investigate differences in common variation across the genome in the susceptibility to the development of PD. 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 ratios <2 [11]. The strongest GWAS associations with PD in European populations are at the SNCA and MAPT locus.

Recently, common variation at the GBA1 locus has been defined as the major risk for PD in African ancestry populations [18]. Although *MAPT* encodes the microtubule associated protein tau, tau pathology is not a ubiquitous feature of PD pathology and the PD causal genes at the *MAPT* locus remain poorly understood. There is some evidence to support the role of genes regulating mitochondrial function at the *MAPT* locus [19]. Annotation of functional pathways from these loci supports the involvement of the lysosomal-autophagy system and immune-inflammatory mechanisms in the pathogenesis of PD [20,21]. Currently, the majority of GWAS have focussed 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.

Neuropathology

Clinico-pathological correlation was used to define the nosology of PD in the last century, and recent advances in structural and biochemical pathology in humans and model systems has led to advances in our understanding of the pathogenesis. LBs and Lewy neurites, first described by Frederic Lewy in 1912, contain the protein alpha-synuclein that forms filaments trapping organelles such as mitochondria and lysosomes [22–25]. Pathogenic mutations in alpha-synuclein lead to accelerated aggregate formation, suggesting that this is an early step in familial, and probably sporadic PD (Figure 1). Pathological alpha-synuclein aggregates at synapses and animal model and human neuropathology suggests that early synaptic dysfunction may be an important step in the pathogenesis of PD [26,27]. Recently, cryo-EM studies have shown that alpha-synuclein filament structure is similar in PD, PDD and DLB while it is different in MSA [24,28].

Alpha-synuclein is a 140 amino acid protein, which contains tandem repeats in the amino terminal part that constitute a lipid binding domain, binding to lipid membranes. Alpha-synuclein is very abundant in the brain where it is found in neurons, and in particular synaptic terminals, and is involved in vesicle transport and neurotransmitter release [28]. The presence of small oligomeric aggregates has also been detected in blood and CSF [29]. LBs represent the end stage of a cellular process in which initial small alpha-synuclein aggregates are observed in the cytoplasm of the neuron, which then coalesce into diffuse pale bodies, followed by the formation of an aggregation seed leading to diffuse synuclein aggregating into filaments that then form a classical Lewy body (Figure 1) [30]. Lewy bodies may not in themselves be detrimental to neurons and most evidence supports the toxicity of small aggregates and oligomers in the pathogenesis of PD, rather than larger aggregates [31].

Using alpha-synuclein staining to detect LBs in post-mortem donors with differing levels of LB pathology, Braak and colleagues proposed a staging of PD, in which the disease could start in the periphery, in the gut or olfactory system, and spread to subcortical and then

cortical brain regions (Figure 1) [30]. The Braak hypothesis is supported by studies of the prodromal phases of PD, including unbiased studies of primary health care records, which show that in the general population those destined to develop motor PD have higher rates of multiple non-motor features including constipation, depression, anxiety, hypotension, urinary and erectile dysfunction [32]. These data have been interpreted as supporting the presence of PD pathology in non-motor regions including the peripheral autonomic nervous system, dorsal motor nucleus of the vagus and the olfactory system before the development of motor disease. However, although the Braak hypothesis is useful in categorising Lewy body pathology in PD, it is clear that many PD cases do not follow the Braak neuropathological staging system [33]. Clinical and neuroimaging studies have suggested that some patients may have brain-stem predominant disease without evidence of peripheral pathology [34]. Furthermore, some patients may 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 [35].

In 2008, the concept of alpha-synuclein spread in the nervous system was supported by direct neuropathological evidence. Clinical trials had been carried out in which the dopaminergic deficit in some PD patients was treated by transplanting human foetal midbrain neurons [36,37]. Autopsies from several graft recipients in these trials showed evidence for LB formation within the intra-striatal transplanted foetal midbrain neurons [36,37]. This strongly suggested that they had developed LBs through spread from the Lewy pathology in the surrounding brain.

The hypothesis of spreading of alpha-synuclein pathology has been validated using several models including injection of preformed alpha-synuclein fibrils into the brain or injection of Adeno-Associated viruses (AAVs) expressing high amounts of alpha-synuclein leading to its aggregation. These spreading studies have been performed in mice, rat and non-human primates and support both the spread from the periphery to the central nervous system and from the brain to other organs [38–41]. Pathological spread is supported by the occurrence of pre-motor gastrointestinal symptoms in patients that may precede the substantia nigra/motor syndrome by decades [32,42]. Specifically, the presence of alpha-synuclein aggregates in neurons surrounding the gut could be the cause of the constipation that has been described in around 20% of patients before motor symptoms. REM sleep behaviour disorder (RBD) is thought to appear when the alpha-synuclein aggregates reach the locus coeruleus and thalamus, which is closely related to the appearance of a movement disorder within the following 10 years [43].

Figure 1 Alpha-synuclein has a central role in the pathophysiology of PD - near here

Molecular mechanisms contributing to PD

Identification of genes that are altered in genetic forms of PD have provided a greater understanding of the molecular mechanisms that contribute to pathobiology [44]. Disruption of inter-organellar homeostasis, impaired mitochondrial and lysosomal function, altered lipid metabolism, ER stress and defective signalling between the ER and mitochondria result in a cascade of events associated with the accumulation of a-synuclein [45,46]**,** deposition of oligomers, fibrils and Lewy bodies/neurite formation which cause synaptic dysfunction and neurodegeneration (Figure 2).

1. Mitochondrial mechanisms

Mitochondria have a fundamental role in the neurodegenerative process of PD. They play key roles in cellular energy production and cell signalling processes that use the cell's bioenergetic status to determine whether the cell survives or undergoes degeneration [47]. Synaptic damage and mitochondrial dysfunction are early events in the pathogenesis of PD and alterations of mitochondrial structure and dynamics are linked to increased production of reactive oxygen species (ROS), abnormal intracellular calcium levels, and reduced mitochondrial ATP production (Figure 2) [48].

Both genetic and environmental factors have been associated with mitochondrial dysfunction in PD pathogenesis. The consistent epidemiological evidence linking pesticide exposure to the risk of PD and the report of a cluster of patients with drug induced parkinsonism related to MPTP toxicity implicates specific toxins in nigral damage, which mediate their effect through mitochondrial complex 1 inhibition [49] (**cite Lancet series PD epidemiology**). The use of neurotoxins to inhibit mitochondrial complex I and induce PD-like syndromes in animal models has provided mechanistic evidence linking mitochondrial dysfunction to PD. A decrease of mitochondrial complex I activity has been reported in the brains of patients with PD and in neurotoxin- or genetic factor-induced i*n vitro* and *in vivo* models [50]. Insights from genetic studies have demonstrated that mutations in 11 genes that can cause parkinsonian syndromes (*SNCA, PRKN, PINK1, DJ-1, LRRK2, ATP13A2, PLA2G6, FBXO7, VPS35, CHCHD2,* and *VPS13C*) alter mitochondrial energy production, ROS production, mitochondrial biogenesis and quality control underpinning a central role for mitochondria in PD [51] .

Neurons, (particularly dopaminergic neurons) have high energy requirements, necessitating high quality mitochondrial bioenergetic function for the normal function and survival of the cells [52,53]. 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 [54]. Two PD genes, PINK1 and Parkin, are involved in mitochondrial quality control in response to overt mitochondrial stress but are not essential

for all types of mitophagy. The LRRK2 G2019S mutation, the most common LRRK2 mutation in PD, was recently shown to disrupt basal mitophagy *in vivo*, indicating that other forms of mitophagy may play a role in neuronal survival [55] Emerging literature indicate that other mitochondrial interactions contribute to the aetiology of PD. For example, it has been reported that aggregated α-synuclein permeabilizes through the mitochondrial membrane and impairs the electron transport chain to enhance oxidative stress-mediated apoptosis in neurons and this may be further exacerbated by hypoxia [56,57]. Mitochondrial dysfunction and the overproduction of reactive oxygen species, facilitates the formation of soluble alpha-synuclein oligomers and insoluble fibrils [58]

Figure 2 Molecular mechanisms contributing to Parkinson's disease -near here

2. 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 PD, a decline in the clearance capacity of the ubiquitin-proteasome and the autophagy-lysosomal systems, together with mitochondrial dysfunction, have been implicated in the pathobiology of PD (Figure 2).

Lysosomes are involved in autophagy and mitophagy and provide pathways to clear abnormal or accumulated proteins [59]. 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 [60].

Insights from genes associated with lysosomal function are mainly provided by mutations in *GBA1*. It has been proposed that heterozygous mutations in *GBA1* 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 [61]. Reduced GCase activity leads to the accumulation of glucosylceramide (GlcCer), which is deacylated by lysosomal acid ceramidase to a toxic metabolite, glucosylsphingosine (GlcSph), and subsequent activation of astrocytes and microglia, releasing pro-inflammatory mediators and causing extensive neuroinflammation [62,63]. There is likely to be further complexity in the association between *GBA1*, lysosomal mechanisms and PD given that non-GD causing mutations are also associated with PD and a recent trial of substrate reduction in PD with venglustat, which successfully lowered the levels of GlcCer had no effect on PD progression.

3. Endocytosis and Cellular trafficking

The involvement of *LRRK2* and *VPS35* in the development of PD 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 ESCRT pathway leading to lysosomal fusion and degradation [64]. LRRK2 is a protein kinase and pathogenic mutations increase the phosphorylation of a series of Rab proteins including Rab-10, 12 and 29. Rab proteins are involved in endocytosis and lysosomal trafficking and the end result of LRRK2 overactivation may be a deficiency in lysosomal function and/or aberrant cellular response to membrane damage [13]. Pathogenic *VPS35* mutations lead to activation of LRRK2, which has also been reported in sporadic (non-monogenic) PD, suggesting that this may be a common pathway in the pathogenesis of PD [64,65].

Immune and Inflammatory mechanisms

It is likely that the host / cellular response in terms of inflammation and immunity are also important mediators of disease progression and pathogenesis (Figure 3). Inflammation was first identified as a component of the neuropathology of PD in the 1980s, when microglial activation [66], and elevated inflammatory cytokines [67] were described in post-mortem PD brain.

Inflammation is well-described not only in the CNS but also in the blood, with low-grade elevation of inflammatory cytokines [68], which importantly has been linked to more rapid disease progression [69]. Monocytes are shifted to a more inflammatory ('classical') phenotype with increased expression of activation markers and proteins associated with cell migration [70]. The neutrophil/lymphocyte ratio is increased [71], and changes in T-lymphocytes include a bias towards pro-inflammatory Th1 and Th17-cell subsets [72], and a reduction in CD8 immunosenescent cells [73]. It is broadly hypothesised that infiltrating immune cells traffic to sites of neuronal damage and contribute, together with local activated microglia, to a chronic neuroinflammatory state. However, immune activation has multifaceted roles, and may confer benefit, particularly in the early stages of neurodegeneration, through promoting clearance of abnormal protein aggregates. Dysfunction of immune-mediated clearance mechanisms may later contribute to aggregate accumulation, although how this protective/toxic balance shifts over time is yet to be fully resolved [74].

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. There is now a well-established link between PD risk and common variation in the Human Leucocyte Antigen (HLA) region [21]. Monogenic causes of PD are also linked to the immune system: *LRRK2* is highly expressed in immune cells [75]. Use of immunosuppressants and corticosteroids was associated with substantially reduced PD risk

in a population-based case-control study of nearly 50,000 incident PD cases and controls [76].

On the background of an immunogenetic predisposition to PD development, a number of factors may drive detrimental immunological and inflammatory responses. Aberrant forms of α-synuclein can trigger an innate immune response via binding to Toll-like receptors (TLRs) on microglial cells and peripheral monocytes [77], as well as inducing a specific adaptive T-cell response [78]. Furthermore, α-synuclein specific T-cell responses in PD 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 PD risk [78]. Mitochondrial dysfunction in PD may also act as a driver of immune activation. Specifically, mitochondrial antigens are presented via MHC class I molecules to CD8 T-cells, a process which is regulated by *Parkin* and *PINK1 [79]*. 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 [80] However, it remains to be established whether mitochondrial autoimmunity plays a role in the pathogenesis of idiopathic forms of PD.

Putative environmental triggers of inflammation include infectious agents entering *via* the gut and nasal routes [81]. Furthermore, recent evidence indicates that α-synuclein may play 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 [82]. Changes in the gut microbiome have also been described with a shift towards over-representation of pro-inflammatory species in PD, producing increased levels of endotoxins and less anti-inflammatory short-chain fatty acids [83]. Gut inflammation may 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; and/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 [84].

Overall, there is abundant evidence for an immune component in PD which 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 PD [85]. T-cell and antibody responses may be dynamic and most prominent in early-stage disease [86,87]. However, evidence around how the immune component of PD evolves over time, and whether the balance of detrimental versus neuroprotective actions varies as a function of disease stage, is lacking. Determining

longitudinal immune changes with greater clarity is essential for planning the optimal timing of clinical trials directed at this highly tractable pathway.

Figure 3 Proposed peripheral – central immune interactions in the pathogenesis of PD Near here

Progression

At a clinico-pathological level the onset of PD 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 PD, most notably REM sleep behaviour disorder patients, almost all disease modifying trials to date have been based around randomisation of a putative therapy to people with early motor disease, with a primary aim of preventing motor progression. Factors determining rapid progression in PD may be particularly important in guiding us to new approaches to disease modification. Conversely, slow progressors may have protective or compensatory mechanisms which may represent targets for disease modification. Currently, important factors implicated in determining the rate of progression include advanced age, impaired lysosomal function, and a proinflammatory state. Candidate gene studies have shown that patients carrying single pathogenic mutations in *GBA1* have a more rapid motor and cognitive progression [88,89]. Genome wide progression studies indicate that ApoE status and related amyloid processing may be important determinant of survival and the development of dementia in PD, highlighting the role of co-pathology [90]. It is likely that vascular and Alzheimer's co-pathology are important determinants of progression in PD associated with dementia (usually multiple protein aggregates can be present in late stage PD associated with dementia) and may be targets for disease modifying therapy in their own right [91].

Pathogenesis driven biomarkers

There are a number of biomarkers which have been used to indicate the extent of neuronal loss and dysfunction, acting as surrogate markers for PD severity and progression. These include functional imaging measures of presynaptic nigro-striatal nerve terminals, and MRI measures of disruption of nigrosomes, atrophy and iron deposition [92], and blood neurofilament light (Nf-L) which is a non specific marker of neuronal damage [91]. However, progress in our understanding of the pathogenesis of PD has led to the development of biomarkers that directly measure certain aspects of PD pathogenesis (Table). Given the likely heterogeneity of PD, these biomarkers may also allow patient stratification according to the predominant underlying mechanism (e.g mitochondrial, lysosomal, immune), enabling targeted patient selection for future clinical trials.

Alpha-synuclein and phosphorylated alpha-synuclein can be directly measured in blood, although there is an overlap between PD patients and healthy controls [91]. Studies of the enhanced aggregation of alpha-synuclein, and of the propagation of Parkinson's pathology, has led to the development of "seeding" assays (real time quaking induced conversion - rtQUIC) based on the ability of patient samples including CSF, skin biopsies, saliva and olfactory biopsies to trigger alpha-synuclein aggregation *in vitro* [93–96]. These assays show promise in improving the early diagnosis of PD, and in distinguishing PD, MSA and unaffected controls. Recently, it has been suggested that these assays may be incorporated in a biological staging system for synuclein disorders encompassing both clinically affected and unaffected individuals, with evidence for a core underlying disease process, analogously to the biological definition of Alzheimer's disease [97,98]. Serum markers of mitochondrial disease have not shown group differences between PD and controls, however in depth analysis of fibroblasts from PD patients suggests that it may be possible to define mitochondrial subgroups of PD patients, highlighting the heterogeneity of PD pathogenesis [99,100]. Magnetic resonance spectroscopy is a promising approach to assessing mitochondrial dysfunction and a relative reduction in ATP can be defined in some PD patients, and this has been incorporated as a secondary outcome measure in trials [101,102].

Specific aspects of lysosomal function have been measured in PD patients, directed by primary genetic aetiology. Bis(monoacylglycerol)phosphates (BMPs) are associated with late endosomal and lysosomal membranes, and change in levels of urinary BMPs have been identified in animal models of LRRK2 dysfunction. LRRK2 G2019S mutation carriers have elevated levels of urinary BMPs presumably reflecting changes in endosomal-lysosomal structure [103,104]. Direct assays of LRRK2 kinase activity can be carried out through cellular assays of Rab proteins which may represent the main physiological substrate for LRRK2 kinases [105,106]. The biochemistry of GBA can be measured through assays of glucocerebrosidase (GCase) activity, GCase protein levels and the levels of the GlucosylCeramide substrate together with other glycosphingolipids affected by changes in GCase. Inflammation in the living PD brain has been demonstrated in PET neuroimaging studies using ligands for the mitochondrial 18-kDa translocator protein (TSPO), which is upregulated in activated glial cells [107]. Cytokine levels in blood and CSF are variable across studies but meta-analysis provides evidence for elevated TGF-beta1, IL-6, and IL-1beta [108].

TABLE near here

Pathogenesis driven drug trials

To date, there are no disease modifying/neuroprotective treatments for PD that have proven effective in Phase 3 clinical trials. However, as outlined here, our rapidly expanding understanding of the pathogenesis of PD has nominated multiple promising drug targets

which might have potential as disease modifying agents and warrant further consideration in early-phase trials (Table) [1]. The concept of total *SNCA* gene expression as a primary driver of disease has led to the development of trials of antisense oligonucleotide (ASO) therapy, which aim to suppress the production of alpha-synuclein in the central nervous system. Anti-aggregation therapy has shown efficacy in transgenic mouse models and at least two agents (anle138b and NPT200-11) have been trialled in Phase 1 studies [1,109]. A number of putative therapies targeting mitochondrial function and oxidative stress have been completed including inosine and coenzyme Q10, which did not meet their primary endpoints. A further Phase 2 trial of ursodeoxycholic acid is ongoing [102]. The emerging data on cell to cell spread of alpha-synuclein pathology has led to treatment trials based on active and passive immunisation against alpha-synuclein. Two recent trials using peripheral administration of monoclonal antibodies against alpha-synuclein did not meet their pre-specified primary endpoints, despite promising preclinical studies [110,111]. Trials of anti-inflammatory agents targeting microglial activation, including minocycline and pioglitazone have failed to show clinical benefit [112,113]. A phase 1 trial has shown that sargramostim (granulocyte-macrophage colony-stimulating factor) boosts T regulatory cell numbers in PD with acceptable safety data [114] . A phase 2 trial of a peripherally-acting immunosuppressant drug, azathioprine, is currently underway [115].

Other trials have been designed to target to specific genes and/or genetic variants[1]. It is unknown whether these agents will be effective in all PD patients, or only in the subset carrying a specific mutation. Most of these studies have included PD patients with both sporadic and monogenic PD. Planned studies directed towards *GBA1* include modulators of GCase including ambroxol and LTI-291 and a *GBA1* gene replacement therapy. A trial of substrate reduction with venglustat was unsuccessful in a Phase 2 study. Finally, *LRRK2* has emerged as a primary target for therapeutic trials with an ongoing trials of an ASO which will suppress the expression of *LRRK2* and of oral LRRK2 kinase inhibitors.

The failure of trials so far may relate to incomplete understanding of the disease pathogenesis, intervention too late in the disease course, incomplete mechanism based patient selection, trials which are too short, or which use clinical outcome measures which do not adequately capture disease progression [1]. Post-hoc analysis based on emerging genetic and pathogenesis markers may provide more information on targeting clinical trials for successful outcomes and the incremental increase in our understanding of PD 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 PD, driven by neurogenetics, have provided insights into the initiation and progression of the disease. PD relates to the formation of abnormal alpha-synuclein aggregates both in the periphery and the brain, and

the spread of this pathology through the brain. This is accompanied by immune activation, neuroinflammation, mitochondrial dysfunction and changes in lysosomal/endosomal function. There are multiple lines of evidence supporting these disparate pathological processes in PD, as well as evidence of overlap / convergence, for example LRRK2 and parkin which appear to be important in endo-lysosomal and mitochondrial function may also play a role in regulating immune responses. The relative importance of these processes is difficult to establish in idiopathic PD. However, in Mendelian forms of PD the primary genetic cause in known and consideration of the different pathological processes in these genetic cases provides some insight into the mechanistic basis of disease heterogeneity. For example, patients with *PRKN* mutations and primary mitochondrial dysfunction have a restricted pattern of cell loss largely confined to the dopaminergic nigrostriatal system, without the widespread pathology and non-motor features seen in typical sporadic PD. Conversely, patients with *SNCA* or *GBA* mutations and prominent alpha-synuclein / Lewy body pathology have early non-motor features including autonomic dysfunction and dementia presumably reflecting widespread pathology throughout the periphery and brain. It is unclear whether these mechanistic and pathological links to clinical heterogeneity seen in genetic forms of PD are also applicable to sporadic, polygenic PD and determining these relationships is a major challenge over the next period in PD research.

In spite of recent advances, many controversies remain unresolved. These include the contribution of peripheral alpha-synuclein pathology to disease initiation; the mechanism by which pathology spreads both from periphery to brain, and within the brain; the size and conformation of alpha-synuclein aggregates which are most neurotoxic; the balance of neuroprotective versus neurotoxic effects of immune activation and how this 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 co-pathologies such as tau, amyloid beta aggregation and vasculopathy in contributing to disease progression. These issues represent key research priorities which need to be addressed through a combination of preclinical research and longitudinal clinico-genetic studies to inform future therapeutic strategies. Despite the complexity and ongoing controversies in the pathogenesis of PD, 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 PD.

Search strategy: We searched PubMed for review articles published in the last 5 years including "Parkinson's" with "Pathogenesis" OR "Genetics" OR "Aetiology" OR "Biochemistry" OR "Pathology" on 26/June/2023. 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 PD, within the space constraints of this review.

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Declaration of interests:

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Morris is a co-applicant on a patent application related to C9ORF72 - Method for diagnosing a neurodegenerative disease (PCT/GB2012/052140)

MGS: MGS is William Scholl Professor with her post supported by the Scholl Foundation endowment to Cambridge University. In the last 12 months she has been paid lecture fees/honoraria -Movement Disorder Society, Institute San Raffaele. She is in the Scientific Advisory Board of the Tau Consortium USA, Qatar Biomedical Research Centre, Fondazione Don Gnocchi, EURAC and EBRI. Consultant for ASTEX and scientific collaboration with Eli Lilly and Teva. Research grants from Parkinson's UK, MJ Fox Foundation, BBSRC, Scholl Foundation, Fondation de la Recherche Alzheimer, Alzheimer's Research UK, MRC, Wellcome Trust, Gates

CMS: is employed by South Eastern Sydney Local Health District, Neuroscience Research Australia and University of New South Wales. She is a National Health and Medical Research Council Practitioner Fellow (APP 1136800). In the last 12 months, she has received research funding from National Health and Medical Research Council of Australia, the Medical Research Futures Fund, Michael J. Fox Foundation for Parkinson's Research (MJFF) and Shake It up Australia and been paid lecture fees/honoraria by the Movement Disorder Society and the Taiwan Movement Disorder Society.

CWG: CWG is employed by the University of Cambridge. In the last 12 months she has received research funding from the UKRI/Medical Research Council, Parkinson's UK, Cure Parkinson's, the Evelyn Trust, and the Cambridge Centre for Parkinson-Plus; consultancy fees from Evidera, Inc.; speaker fees from GSK and the World Parkinson Congress.

Author Contributions:

HM did the literature search prepared the first and final draft, wrote the Introduction, Clinical, Genetics, Pathogenesis driven and Conclusions sections and prepared the table. MGS prepared the Neuropathology section and Figure 1. CMS prepared the Mitochondrial, Proteostasis and endocytosis sections and prepared Figure 2. CWG wrote the Immune and inflammatory mechanisms sections and wrote the Pathogenesis driven and Conclusions sections. All authors contributed to writing the final version of the paper and approved the final version for publication.

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Legends to Figures - The pathogenesis of Parkinson's disease

Figure 1 Alpha-synuclein has a central role in the pathophysiology of PD

Natively unfolded alpha-synuclein aggregates into oligomers and fibrils, forming the characteristic Lewy bodies and Lewy neurites. Lewy body pathology is found in the gut, peripheral nervous system and brain and the "spreading" theory of PD pathology suggests that alpha-synuclein aggregation spreads between contiguous regions. Lewy body and neurite formation is associated with cell loss and dysfunction in the substantia nigra. Created with BioRender.com.

Figure 2 Molecular mechanisms contributing to Parkinson's disease

Two major contributors to the pathogenesis of Parkinson's disease include mitochondrial dysfunction and impaired protein clearance pathways. Loss of mitochondrial function can by caused by PD gene mutations (e.g in *PKN, PINK1, DJ1*) or mitochondrial damage from toxins or biological aging. As a consequence, reduced cellular ATP, increased ROS production and alterations in calcium release cause synaptic dysfunction and neurodegeneration. Protein clearance pathways, particularly the autophagy-lysosomal system (rather than the ubiquitin-proteasome system) can be impaired (by e.g GBA or other PD gene mutations and lead to progressive a-synuclein aggregation, accumulation, Lewy body/formation and neurodegeneration. Molecular interactions between these pathways can occur to accelerate the aggregation of a-synuclein (e.g mitochondrial dysfunction impairs lysosomal function to clear aggregated proteins; aggregated a-synuclein may cause mitochondrial dysfunction),

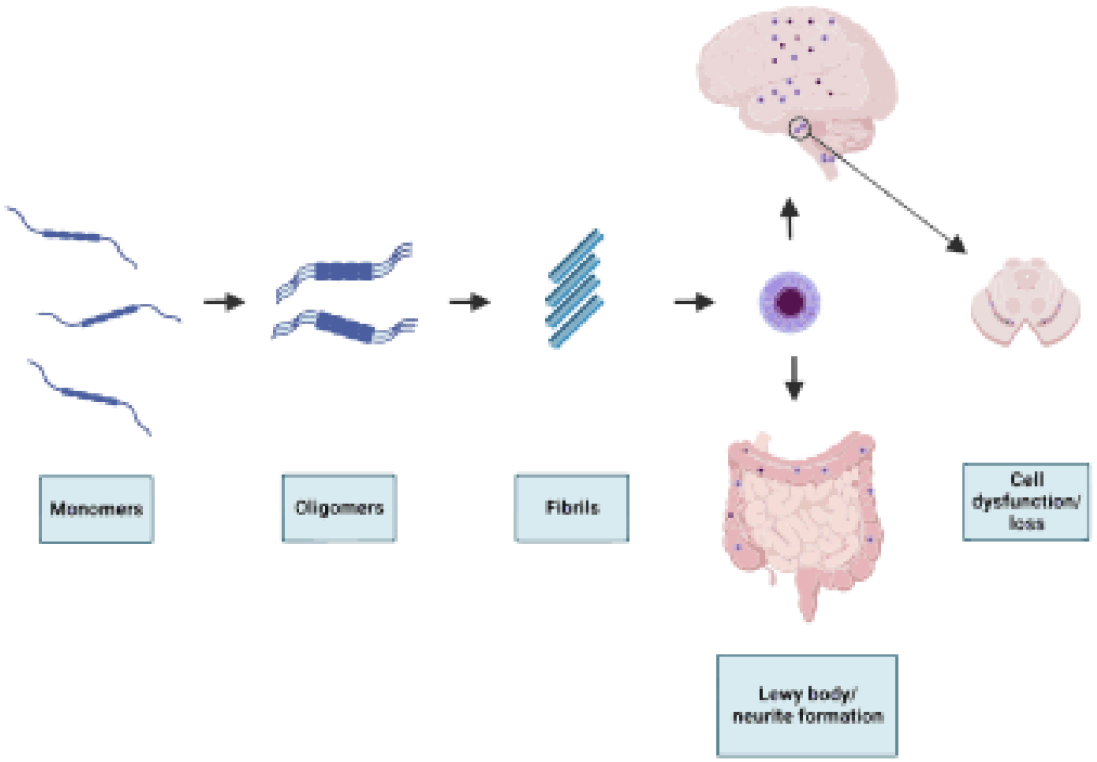
setting up vicious cycles that lead to neurodegeneration in Parkinson's disease. Created with BioRender.com.

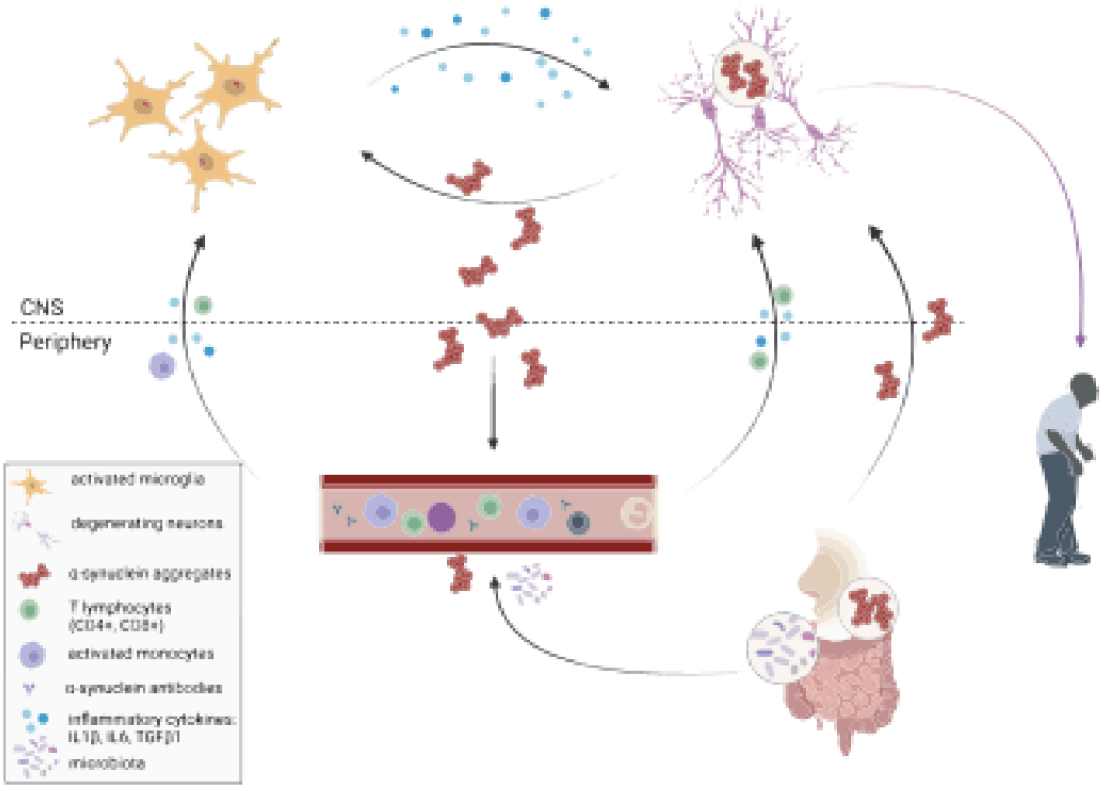
Figure 3 Proposed peripheral – central immune interactions in the pathogenesis of PD

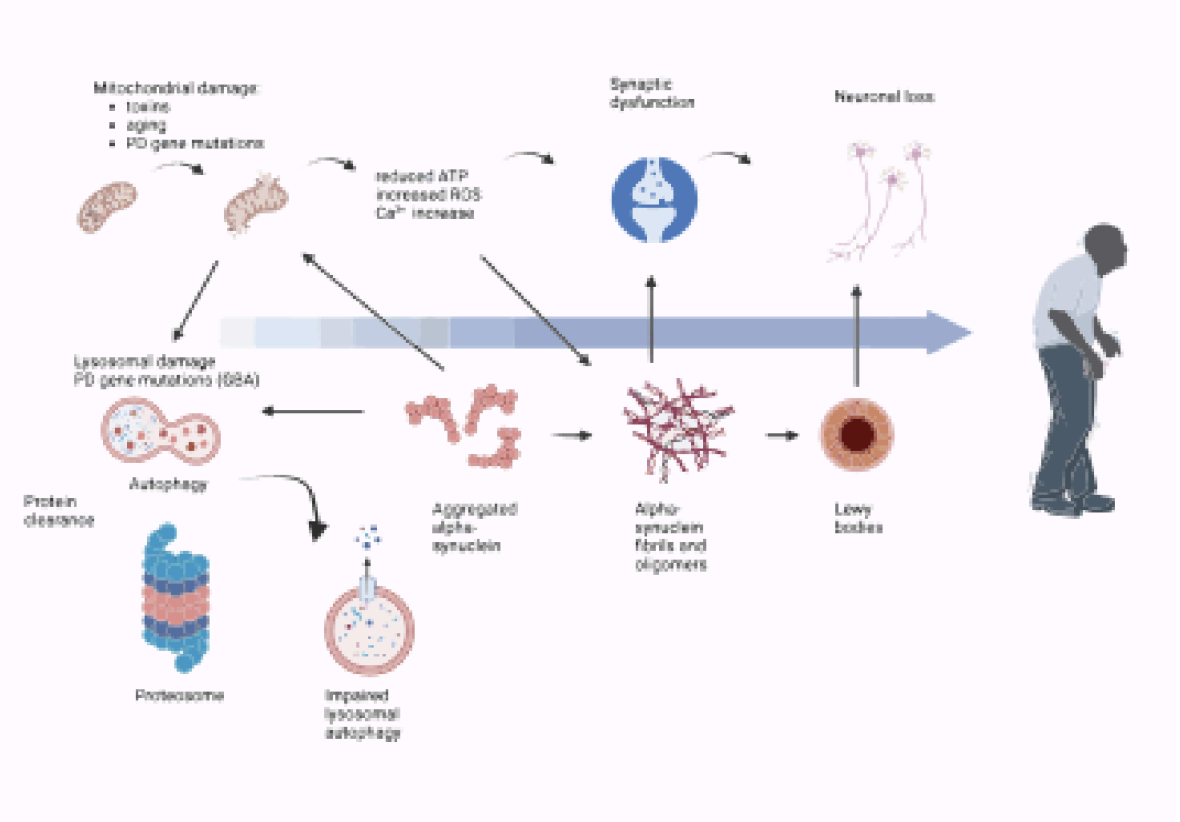
Alpha-synuclein aggregates form at an early stage in enteric and olfactory nerves, with aggregation possibly exacerbated by local infections. These aggregates may spread via the vagal/olfactory nerves to the brain where they have a neurotoxic effect, leading to cell degeneration and death. Alpha-synuclein oligomers and other toxic species released from dying cells promote local microglial activation within the brain, which may initially be beneficial though phagocytosis of cell debris and toxic proteins, but ultimately becomes pro-inflammatory, with the release of neurotoxic cytokines. Alpha-synuclein oligomers may leak into the peripheral blood from the brain via the glymphatic system, or from peripheral tissues including the gut and olfactory system. These oligomers can drive immune activation in the periphery, including an adaptive alpha-synuclein specific immune response. Bacterial endotoxins released from the gut in association with a dysregulated microbiome also contribute to peripheral inflammation and immune activation. Inflammatory cytokines (including IL1β, IL6, TGFβ1) and peripheral immune cells (CD4+ Th1, CD4+ Th17 and CD8+ T lymphocytes including alpha-synuclein specific T-cells, and activated monocytes) traffic across the blood-brain barrier to sites of neurodegenerative alpha-synuclein pathology, where they promote further microglial activation as well as having a direct neurotoxic effect. Created with BioRender.com.

Mechanism	Proposed Pathogenesis	Genetic evidence	Biomarkers	Therapeutic implications
Increase in *SNCA* expression	Increase in alpha-synuclein protein leads to increased aggregation and cell death and dysfunction	Increased *SNCA* gene dosage (duplications/triplications) cause PD; *SNCA* common variants likely lead to increased expression	Alpha-synuclein and phospho-synuclein measurement in blood	Decrease in *SNCA* transcription or translation e.g. with ASO therapy
Increase in *alpha-synuclein* aggregation	Formation of oligomers and fibrils lead to cellular toxicity	Coding mutations in *SNCA* lead to increase in alpha-synuclein aggregation	rt-QUIC assays for aggregation based on CSF, skin and olfactory mucosal biopsies	Anti-aggregation therapies
Mitochondrial dysfunction	Reduced complex 1 activity, abnormal calcium homeostasis, increased reactive oxygen species, reduced mitochondrial ATP production	Multiple PD gene mutations lead to changes in mitochondrial function including PRKN, PINK1 and LRRK2	Magnetic resonance spectroscopy analysis of Pi/ATp ratios; Measurement of ATP and mitochondrial function in skin biopsies	Enhancing mitochondrial biogenesis and function
Altered endosomal-lysosomal trafficking	Activation of LRRK2 and VPS35 lead to phosphorylation of RAB proteins leading to decreased lysosomal function; and altered response to membrane damage	Rare pathogenic variants in LRRK2 (e.g. G2019S) and VPS35 lead to increased RAB phosphorylation	Measurement of Rab protein phosphorylation in cells from peripheral blood.	Reducing LRRK2 protein levels and/or kinase activity with ASO therapy or kinase inhibitors
Lysosomal dysfunction	Impaired alpha-synuclein degradation leading to increased cellular alpha-synuclein	GBA1 mutations are associated with PD; and rare variants in other genes may be relevant	Measurement of GCase protein and enzyme activity. Measurement of GSLs in blood and CSF.	Modulators of Beta-glucocerebrosidase activity
Immune activation and neuroinflammation	Multiple factors (alpha-synuclein aggregates, mitochondrial antigens, gut bacterial endotoxins) promote both innate and adaptive immune responses culminating in increased neuroinflammation and neuronal toxicity.	Association between HLA variants and PD; LRRK2, PRKN and PINK1 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 alpha-synuclein spread between anatomically contiguous cells; or over	NA	rt-QUIC assays for aggregation based on CSF, skin and olfactory mucosal biopsies	Reduction in release, extracellular transit or uptake by recipient cells using monoclonal antibody or other

	longer range; may be contained in extracellular vesicles			therapies


Monomers
Oligomers
Fibrils
Cell dysfunction/ loss
Lewy body/ neurite formation


CNS
Periphery
activated microglia
degenerating neurons
α-synuclein aggregates
T lymphocytes (CD4+, CD8+)
activated monocytes
α-synuclein antibodies
inflammatory cytokines: IL1β, IL6, TGFβ1
microbiota


Mitochondrial damage
• toxins
• aging
• PD gene mutations
reduced ATP
increased ROS
Ca2+ increase
Synaptic dysfunction
Neuronal loss
Lysosomal damage
PD gene mutations (GBA)
Autophagy
Protein clearance
Aggregated alpha-synuclein
Alpha-synuclein fibrils and oligomers
Lewy bodies
Proteasome
Impaired lysosomal autophagy