PARKINSON DISEASE

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Abstract | Parkinson Disease (PD) is the second most common neurodegenerative disorder affecting between 2-3 % of the population aged 65 or older. Neuronal loss in the substantia nigra, causing striatal dopamine deficiency, and intracellular inclusions containing aggregates of alpha-synuclein are the neuropathological hallmarks. Multiple other cell groups throughout the central and peripheral autonomic nervous system are also involved, probably already in early disease. Although clinical diagnosis rests on the presence of bradykinesia and other cardinal motor features PD is associated with a plethora of non-motor symptoms adding to overall disability. The underlying molecular pathogenesis of PD involves multiple pathways and mechanisms including α-synuclein proteostasis, mitochondrial function, oxidative stress, calcium homeostasis, axonal transport and neuroinflammation. Recent research into diagnostic biomarkers has taken advantage of neuroimaging where multiple modalities, including PET and SPECT and novel MRI techniques, have been shown to aid early and differential diagnosis. Treatment of PD is anchored on pharmacologial substitution of striatal dopamine supplemented by non-dopaminergic approaches to address both motor and non-motor symptoms and deep brain stimulation (DBS) for those developing intractable L-Dopa related motor complications. Experimental therapies have tried to restore striatal dopamine by gene- and cell-based approaches and most recently aggregation and cellular transport of α-synuclein have become therapeutic targets. One of the greatest current challenges in PD research is to identify markers for prodromal disease stages, which would allow novel disease-modifiying therapies to be started earlier.

[H1] Introduction

200 years after James Parkinson's seminal essay on 'the shaking palsy' most of his original clinical observations have stood the test of time. Beyond perception of Parkinson disease (PD) as a disorder of movement it has since become apparent that a multitude of non-motor features, such as cognitive impairment, autonomic dysfunction, disorders of sleep, depression and hyposmia, are part of the disease and add significantly to overall burden. Tremendous progress has been made in understanding the neuropathology and its progression throughout the nervous system as well as the molecular and neurophysiological mechanisms and perturbations underlying the disease and its symptoms. Above all, highly efficacious therapies have become available, centered around pharmacological dopamine substitution, but with important refinements and ground-breaking expansions like the introduction of deep brain stimulation (DBS). While this has undoubtedly made PD the first and still unparalleled example of a neurodegenerative disease which can be effectively managed leading to sustained symptom control and quality of life over up to decades, PD remains a progressive disease eventually causing severe disability – not least by the increasing severity of treatment-resistant motor problems like postural instability and falling in combination with non-motor symptoms like cognitive decline and autonomic failure. Modifying disease progression and further delaying disability thus are the key unmet needs to be addressed by current and future research efforts. Of great future potential is the development of methods to identify individuals at risk and early manifestations that antedate the onset of the defining motor symptoms.

In this Primer we describe the epidemiology of PD, review our current understanding of the underlying pathology and molecular pathogenesis as well as the perturbations of basal ganglia – cortical connectivity that underlie the cardinal motor features of this illness. We

also summarize recent advances in clinical diagnostics, biomarker research and screening, provide an overview of the natural history of PD and current, as well as future, therapies.

[H1] Epidemiology

PD is rare before age 50, and increasingly common in each subsequent decade.[1] Worldwide incidence estimates range from 5/100,000 to over 35/100,000 new cases yearly[1], likely reflecting differences in the demographics of the populations studied or in study methods. In a population-based study in Minnesota (USA) with pathologic validation of clinical diagnoses, PD incidence was 21 per 100,000 person-years[2]. PD incidence increases 5 to 10 fold from the sixth to the ninth decade of life[1-3] and PD prevalence, conservatively estimated at 0.3% overall, likewise increases sharply with age, to more than 3 % in those over age 80 (Fig. 1).[4]

Mortality is not increased in the first decade after the onset of PD, but increases thereafter, eventually doubling.[5] Improvements in health care and thus longer survival were associated with increasing PD prevalence over time in one 20 year study.[6] The number of people with PD is expected to double between 2005 and 2030.[7] Years lived with disability and disability adjusted life years due to PD increased between 1990 and 2010, and a progressive increase in the societal and economic burden of PD is expected in the future as the world population ages.[7-9] Costs associated with PD will rise, along with the societal loss and the personal burden of lost health and, for many, lost independence[10].

PD is twice as common in men than women in most populations[3,11], although in a few populations, including one study from Japan, no difference or even a female excess was observed[12]. A protective effect of female sex hormones, a sex-associated genetic mechanism, or sex-specific differences in exposure to environmental risk factors may explain this male preponderance, although disparities in health care may also contribute.

PD incidence appears to vary within subgroups defined by race, ethnicity, genotype or environment. PD may be less common in African Americans and Asians in the U.S., but systematic race-specific incidence has not been investigated in other multiracial populations, and societal rather than biological causes may underlie these findings[3] . Geography and race are often related, and it may be difficult to determine the relative contribution of each to PD risk. In Israel, PD prevalence is high, possibly reflecting the higher prevalence of the incompletely penetrant PD-associated genes, *LRRK2* and *GBA*, in Ashkenazi Jews[13]. The PD prevalence is also high in Inuit, Alaska Native and Native American populations[14]. Lifestyle, including dietary exposure to persistent organic pollutants, or shared genetic factors may explain this pattern. PD incidence is greater in men of Japanese and Okinawan descent living in Hawaii than in men living in Japan, supporting that environmental factors play a role[15]. Gene-environment interactions definitely modify the risk for sporadic PD. For example, PD incidence is significantly greater in persons exposed to certain environmental factors, such as pesticides and traumatic brain injury, and lower in smokers or caffeine users[16].

[H1] Mechanisms/pathophysiology

[H2] Neuropathology

Characteristic features of PD include neuronal loss in specific areas of the substantia nigra and widespread intracellular protein (α-synuclein) accumulation. Although neither the loss of pigmented dopaminergic neurons in the substantia nigra[17,18], nor the deposition of α-synuclein in neurons is specific for PD, these two major neuropathologies are specific for a definitive diagnosis of idiopathic PD when applied together (Fig. 2).

Gross macroscopic atrophy of the brain is not a feature of PD, rather neuronal degeneration occurs in only certain types of neurons within particular brain regions. In early stage disease loss of pigmented dopaminergic neurons is restricted to the ventrolateral substantia nigra with relative sparing of other midbrain dopaminergic neurons (Fig. 2A-D)[19,20] but becomes more widespread by end-stage. The dramatic loss of these dopaminergic neurons even early in the disease suggests that the degeneration in this region starts before onset of motor symptoms, which is supported by a number of recent clinicopathological studies[21,22].

The other required neuropathology is the abnormal deposition of α-synuclein in the cytoplasm of certain neurons in several different brain regions[23]. Lewy bodies, which are largely made up of aggregated α-synuclein, were the first to be described over a century ago. Following the development of refined histopathological methods, a broader range of α-synuclein aggregates have been described (Fig. 2E-G). The Lewy pathology initially occurs in cholinergic and monoaminergic brainstem neurons and neurons in the olfactory system, but are also found in limbic and neocortical brain regions with disease progression (Fig. 2H). In patients with Alzheimer pathology, there is a different pattern of α-synuclein pathology that concentrates mainly in limbic brain regions[22].

[H2] Molecular pathogenesis

Although heritable forms of PD only represent 5-10% of all cases (table 1), some of the mutant genes regulate a set of molecular pathways that when perturbed can trigger a neuropathology that resembles, or is indistinguishable, from idiopathic PD. Large genome-wide association studies (GWAS) suggest that that genes encoding for proteins involved in these molecular pathways can play a role also in sporadic PD[24]. Examples of these pathways are: α-synuclein proteostasis, mitochondrial function, oxidative stress, calcium homeostasis, axonal transport and neuroinflammation (Fig. 3).

[H3] α-synuclein proteostasis

Intraneuronal protein aggregates largely made up of α-synuclein are found in all PD cases. The normal neuronal function of the 140 amino acid α-synuclein protein is not fully understood, but it occurs in the cytosol, possibly also in mitochondria and the nucleus, and likely plays a role in synaptic vesicle dynamics, but has also been implicated in mitochondrial function, intracellular trafficking and as a potential chaperone[25-27] During a pathogenetic process when soluble α-synuclein monomers initially form oligomers, then progressively combine to form small protofibrils and eventually large, insoluble amyloid fibrils (i.e. the "aggregates" that make up Lewy pathology), they acquire neurotoxic properties[28,29]. The underlying triggers of accumulation and aggregation of α-synuclein can be manifold, e.g. a relative overproduction of the protein or the presence of mutations that increase the likelihood for its misfolding and oligomerization, or impairments in molecular pathways

charged with degrading α-synuclein, either in its native state or after it has misfolded. The aged brain is particularly susceptible, and it can be presumed that this is at least partly due to a progressive, age-related decline in proteolytic defense mechanisms. The existence of point mutations in *SNCA*, the gene encoding α-synuclein, that cause PD, as well as families with multiplications of the *SNCA* that develop a PD-like condition, strongly support the notion that α-synuclein is a key player in PD. Similarly, GWAS have revealed a single nucleotide polymorphism associated with the *SNCA* locus that alter risk for sporadic PD and which is associated with increased expression levels of α-synuclein[24,30]. A study in human iPSC-derived neurons and frontal cortex from PD patients supports the idea that a PD risk variant in a non-coding distal enhancer element of *SNCA* is coupled to increased α-synuclein expression[25].

[H3] α-synuclein degradation. Intracellular homeostasis of α-synuclein is maintained by the actions of the ubiquitin proteasome system (UPS) and the lysosomal-autophagy system (LAS). The relative importance of UPS and LAS for intracellular α-synuclein proteolysis in neurons is debated and LAS appears more central than the UPS to clearance of oligomeric assemblies[31] . Additional proteases, that are not part of UPS and LAS, can cleave α-synuclein also in the extracellular space[31]. Both chaperone-mediated autophagy (CMA) and macroautophagy are suggested to mediate α-synuclein degradation[31,32]. CMA involves specific chaperones which target certain proteins to lysosomes whereas macroautophagy entails formation of vesicles with double membranes, called autophagosomes that are directed to perinuclear lysosomes. Inhibition of either system leads to increased α-synuclein levels and there is evidence for some compensatory cross-talk between the systems[33].

Importantly, increasing age, the greatest risk factor for PD, is associated with reduced LAS and UPS functions[34] which is consistent with observations of increased basal levels of α-synuclein in nigral dopamine neurons during normal ageing[35]. Pharmacological stimulation of macroautophagy reduces cellular α-synuclein levels in experimental models[36,37]. In PD patient substantia nigra and experimental PD models, lysosomal enzymes are reduced particularly in cells containing α-synuclein inclusions[38], markers of CMA are decreased[39] and autophagosomes accumulate[40]. Additional observations support the idea that altered proteostasis profoundly influences neuronal accumulation of α-synuclein. For example, α-synuclein oligomers inhibit UPS[41], accumulating α-synuclein can inhibit macroautophagy[42,43] and different forms of α-synuclein (wild type, mutant and dopamine-modified) can reduce CMA function[33,44]. Collectively, these observations suggest a vicious cycle involving accumulation of α-synuclein and failure of neuronal proteostasis.

Several mutations associated with familial PD also reduce LAS function. One consequence of expression of *G2019S* mutation in the gene encoding for LRRK2 is associated with impaired LAS and increased aggregation of α-synuclein in dopamine neurons exposed to α-synuclein fibrils[45]. Heterozygous mutations in the gene encoding the lysosomal enzyme glucocerebrosidase (*GBA*) constitute the most common genetic risk factor for PD[46] and are also coupled to reduced LAS function[47]. GWAS has revealed two polymorphisms in the *GBA* locus associated with altered PD risk[24], and normal aging is reported to result in a progressive decline in GBA activity[48]. Recent evidence from clinical cohort studies also suggests an increased risk for dementia in PD subjects carrying GBA mutations that in the homozygotic state are associated with the neuronopathic from of Gaucher disease[49,50]. Reduced GBA activity coincides with increased α-synuclein levels both in cell cultures and

animal models[51,52]. Mutations in the gene encoding vacuolar protein sorting-35 (*VPS35*) which cause autosomal dominant PD[53,54] also appear to affect α-synuclein handling. VPS35 is part of the retromer complex which plays a key role in sorting lipids and proteins that are newly synthesized or have undergone endocytosis, and directs them to either the lysosome, cell surface or Golgi[55]. Notably, *Vps35*-deficient mice exhibit increased α-synuclein levels in nigral dopamine neurons[56], while overexpression of *Vps35* reduces α-synuclein accumulation in transgenic mice overexpressing α-synuclein and in cultured neurons exposed to α-synuclein fibrils[57]. Both VPS35 deficiency and the D620N mutation in *VPS35* that causes PD are coupled to reduced cellular levels of lysosomal protein LAMP2[56], suggesting once again that LAS perturbation is key to PD pathogenesis. Finally, mutations in the *ATP13A2* (PARK9) gene, which encodes a type 5 P-type ATPase that is present in lysosomes and autophagosomes[58], are associated with a rare juvenile-onset neurological condition (Kufor-Rakeb syndrome) that includes parkinsonian features and responds to dopaminergic therapy[58]. Dysfunction of LAS and vesicular trafficking likely contribute to neurodegeneration in people with mutations in *ATP13A2*[59]. Notably, GWAS have revealed that certain *ATP13A2* variants are associated with increased penetrance of *LRRK2* mutations and heightened PD risk in *GBA* mutation carriers, which supports the idea that these genes act in shared molecular pathways[60].

[H3] Prion-like propagation of α-synuclein pathology

An additional mechanism for the development of α-synuclein aggregates has recently been proposed. The prion-like hypothesis for α-synuclein posits that once α-synuclein aggregates have formed in a neuron they can be transported intra-axonally to other brain regions, be released into the extracellular space, be taken up by neighboring neurons and seed aggregation of endogenous α-synuclein once inside their new cellular host[61,62]. Cell culture studies have demonstrated that LAS impairment leads to increased secretion of α-synuclein into the extracellular space through exosomes, and that endocytosis is a key mechanism of uptake of extracellular α-synuclein, which is in part mediated by a transmembrane protein called lymphocyte-activation gene 3[63,64] . Thus, initial α-synuclein misfolding in a small number of cells could progressively lead to the spread of α-synuclein aggregates to multiple brain regions over years or decades following the initial insult. This is consistent with the idea that α-synuclein pathology gradually engages more brain regions as the disease progresses, as suggested by Braak and others[23]. In addition, this model supports the idea that the first sites of α-synuclein aggregation might be in the gut enteric nerves and the olfactory bulb where they underlie the symptoms and signs associated with prodromal PD (e.g. anosmia, constipation)[65,66], before they spread, eventually leading to motor dysfunction once the substantia nigra has become involved[67].

[H2] Mitochondrial dysfunction

Several lines of evidence have implicated mitochondrial dysfunction as a key element in the pathogenesis of PD (reviewed in detail in references[68,69]). An emerging picture is one of a vicious cycle where α-synuclein aggregation and mitochondrial dysfunction exacerbate each other, which could explain why these cellular changes are observed together in degenerating neurons in PD.

Mitochondrial complex 1 activity is reduced in cells from various tissues of PD patients[68,69] Peroxisome proliferator-activated receptor gamma (PPARγ coactivator-1 (PGC-1α), a mitochondrial master transcriptional regulator, target genes are generally underexpressed in

PD[70]. It has been proposed that low levels of α-synuclein are normally present in mitochondria, but that accumulation of the protein inside mitochondria leads to complex 1 deficits and oxidative stress[71]. Activation of PGC-1α results in reduced α-synuclein oligomerization and toxicity *in vitro*, whereas induced PGC-1α deficiency by genetic knockdown increases vulnerability to α-synuclein oligomers[72]. Conversely, exposure to α-synuclein oligomers reduces cellular PGC-1α levels[72]. It has been proposed that low levels of α-synuclein are normally present in mitochondria, but that accumulation of the protein inside mitochondria leads to complex 1 deficits and oxidative stress[71]. In animal models, injection of several toxins that impair mitochondrial function replicate features of PD neuropathology[68,69]. When mitochondrial transcription factor A, which is essential for mitochondrial DNA expression, is selectively depleted in dopamine neurons of mice (so called MitoPark mice), nigral dopamine neurons exhibit a defective respiratory chain and degenerate in adulthood[73]. Adult mice which lack one allele of the *Engrailed1*, which enhances nuclear translation of the mitochondrial complex proteins NDUFS1 and NDUFS3, replicate several important features of PD neuropathology, e.g. perturbations of autophagy, neuroinflammation and progressive nigral dopamine neuron death following retrograde axonal degeneration[74]. Importantly, axonal degeneration, potentially due to the energy deficiency, might be an upstream and early neurodegenerative event in PD. Human brain imaging studies have demonstrated changes in the striatum in people even several years before they are diagnosed with PD[75,76], and recent post-mortem studies suggest that nigrostriatal terminals are dysfunctional or have degenerated several years before the neuronal cell bodies in the substantia nigra die[77]. An alternate explanation for the axonal degeneration is that α-synuclein aggregates eventually become obstacles to normal axonal transport[78].

Recent advances in the understanding of molecular pathways governed by PD genes have provided additional support to the notion that mitochondrial failure is a key event in disease process. For example, *LRRK2* mutations are not only associated with changes in autophagy, but also with mitochondrial impairments[69]. Moreover, proteins encoded by *parkin* and *PINK1*, recessive PD genes, cooperate in the clearance of damaged mitochondria through mitophagy[79]. Impaired degradation of Miro (a protein in the outer mitochondrial membrane that connects the organelle to microtubule motors) seems to play a role in defective clearance of damaged mitochondria. In iPSC-derived neurons from both inherited and sporadic PD, degradation of Miro is reduced and as a consequence mitophagy is inefficient which ultimately could lead to energy failure[80].

[H2] Oxidative stress

Evidence that oxidative stress, as a consequence of mitochondrial dysfunction, is increased in PD brain tissue is compelling[81], but it is debatable whether it occurs early or late during the demise of neurons. Mutations in DJ-1, a putative antioxidant, which cause early-onset autosomal recessive PD[82], are associated with increased cellular oxidative stress[83,84]. Knocking out DJ-1 results in increased protein oxidation in stressed nigral dopamine neurons.

Nigral dopamine neurons have been suggested to be particularly vulnerable to metabolic and oxidative stress for several reasons. First, they possess particularly long (up to 4.5 meters) and unmyelinated axons, with large numbers of synapses (estimated at 1-2.4 million per nigral dopamine neuron) which require great energy to be sustained[85,86]. Second, they

(unlike the dopamine neurons in the neighboring ventral tegmental area which are relatively resilient in PD) exhibit autonomous pacemaking activity involving the cytosolic calcium oscillations and calcium extrusion at the expense of energy[87,88]. Third, elevated levels of cytosolic dopamine and its metabolites can cause toxic oxidative stress[89,90]. As a final note, mitochondrial dysfunction and increased oxidative stress can lead to depletion of lysosomes[91] and functional impairment of LAS, further demonstrating that several putative pathogenic pathways in PD are intimately linked.

[H2] Neuroinflammation

A large number of post-mortem, brain imaging and fluid biomarker studies shows that neuroinflammation is a salient feature of PD[92]. Although maybe not the initial trigger, neuroinflammation likely is an essential contributor to PD pathogenesis[93,94]. Catecholaminergic neurons in PD brains and cultured dopamine neurons (when exposed to activated microglia or L-dopa) have been reported to be particularly inclined to express major histocompatibilty complex class I (MHC- I) antigens, which exposes them to cytotoxic T-cell mediated death if they present foreign protein[95].

GWAS indicate that PD-associated genes often encode for proteins expressed in immune cells and involved in immune regulation, such as LRRK2 (involved in autophagy by immune cells)[24,96,97]. There are close links between certain PD genes, protein aggregates and neuroinflammation. Evidence from patients and experimental models suggest that α-synuclein aggregation induces both innate and adaptive immunity in PD[93,94], and neuroinflammation can also promote α-synuclein misfolding[98], suggesting that the two processes participate in a self-aggravating cycle. During prodromal PD, tissue inflammation in the olfactory system or gut has been suggested to trigger a sufficient level of α-synuclein misfolding that some α-synuclein aggregates eventually escape the normal degradation mechanisms[99]. Indeed, recent evidence from experiments in αSyn overexpressing mice suggest a role of gut microbiota in promoting microglial activation and αSyn pathology as well as motor deficits[100].

It would be misleading, however, to suggest that activated immune cells only contribute to the initiation or deterioration of PD pathology in the brain. Microglia can phagocytose and degrade extracellular α-synuclein aggregates and immunotherapies that target α-synuclein, and which are currently being developed for clinical trials, rely upon the clearance of antibody-bound α-synuclein by activated immune cells[101].

[H2] Motor circuit pathophysiology in PD

The basal ganglia are part of several parallel, but anatomically segregated thalamo-cortico-basal ganglia circuits, which have important functions in the control of actions and goal-directed behavior. These circuits are anatomically characterized by a strong convergence of cortical input onto relatively few subcortical output neurons and back to cortex suggesting a "filter like" function. Four circuits with a functionally similar, yet topographically distinct organization have been identified to subserve limbic, prefrontal-associative, oculomotor and motor functions by linking the corresponding frontal cortical areas and subregions of thalamus and basal ganglia (shown in Fig. 4 for the motor circuit) [102,103].

Parkinsonism results from a decreased dopaminergic transmission in the motor region of the striatum with opposing effects on the direct and indirect pathway resulting in increased GABAergic inhibition of thalamo-cortical projections. (Fig. 4b). This firing rate model, provided a rationale for the renaissance of stereotactic surgery in PD in the early 1990s, because akinesia was no longer considered a "loss of function" symptom, but rather the physiological consequence of increased inhibitory output activity of the basal ganglia. Indeed, lesioning of the internal globus pallidus or subthalamic nucleus proved effective in alleviating bradykinesia in animals and humans[104,105]. Meanwhile the model has been amended by additional connections such as the "hyperdirect pathway", a monosynaptic link between motor cortical areas and the subthalamic nucleus, which changed the perception of the subthalamic nucleus from a passive relay nucleus to a second input structure of the basal ganglia (Figure 4)[106]. The hyperdirect pathway may play a role in preventing premature responses by reinforcing indirect pathway activity and thereby the "breaking" function of the basal ganglia, thus allowing more time for the selection of the most appropriate response at the cortical level[107]. Moreover, recent animal studies have suggested, that antidromic activation of the hyperdirect pathway may drive the strong anti-akinetic effect of subthalamic nucleus deep brain stimulation, further underlining the functional significance of this second basal ganglia input[108,109].

Changes in firing rate, however, are not capable of fully explaining the pathophysiology of hyper- or hypokinetic movement disorders. Growing evidence suggests that movement disorders are characterized by more complex changes in information processing such as abnormal neural synchronization and cortico-subcortical coupling in specific frequency bands as indexed by EEG power density and spectral coherence. The Parkinsonian off-state is characterized by enhanced beta band activity (~20 Hz) in local field potential recordings from the basal ganglia, which is suppressed by dopaminergic medication or deep brain stimulation in parallel with the clinical improvement of bradykinesia and rigidity[110,111]. In contrast, hyperkinesia - such as levodopa-induced dyskinesia in PD - have been associated with increased theta band activity in the same structures (4 - 12 Hz)[112]. High-frequency deep brain stimulation suppresses either activity and may thus act like a "filter" for abnormally synchronized basal ganglia activity, irrespective of the underlying disorder.
In addition, changes in cerebellar activity and the interaction between basal ganglia and cerebellum may be important for the pathophysiology of tremor in PD[113] and disorders of balance and gait likely involve abnormal basal ganglia output via projections into the midbrain locomotor region (pedunculopontine and cuneiform nucleus)[114]. A better understanding of this expanded motor network may help to define alternative targets for DBS in PD targeting specific symptom profiles.

[H1]Diagnosis, screening and prevention

[H2] Clinical diagnosis and natural history

PD is clinically defined by the presence of bradykinesia and at least one additional cardinal motor feature (rigidity or rest tremor) as well as additional supporting and exclusionary criteria[115-118]. Onset of motor symptoms is usually unilateral and asymmetry persists throughout the disease. The average age of onset of PD is in the late fifties, with a broad range from less than 40 to over 80 years. Young-onset PD is commonly defined by an age of onset below 45 and more than 10% of those subjects have a genetic basis, the latter being found in more than 40% of those with even earlier onset before age 30[119,120].

In addition to the cardinal motor features, a majority of PD patients also suffer from non-motor symptoms[121] adding to the overall burden of parkinsonian morbidity (Figure 5). Non-motor symptoms (NMS) in PD involve a multitude of functions including disorders of sleep-wake cycle regulation, cognitive impairment (frontal executive dysfunction, memory retrieval deficts, dementia and hallucinosis), disorders of mood and affect, autonomic dysfunction (orthostatic hypotension, urogenital dysfunction, constipation and hyperhydrosis) as well as sensory symptoms (most prominently hyposmia) and pain[121]. Some of these, e.g. constipation, olfactory loss, anxiety, depression and RBD, may antedate the onset of classical motor symptoms by years or even decades (see below). NMS become increasingly prevalent and obvious over the course of the illness and are a major determinant of quality of life, progression of overall disability and of nursing home placement in PD[4]. In one long-term series dementia was present in 83%, hallucinosis in 74%, symptomatic orthostatic hypotension in 48%, constipation in 40% and urinary incontinence in 71% of those surviving for more than 20 years. Progressive disability from PD in that series ultimately included treatment resistant motor symptoms like freezing of gait (81%) postural instability and falling (87%, with fractures in 35%) and choking (48%).[122]
While these milestones of progression are key events in the long-term evolution of PD clinical trials and observational studies so far have focused on progression of motor impairment as captured by the Unified Parkinson's Disease Rating Scale (UPDRS) which is the most commonly used scale to monitor PD-related motor disability in research settings[123,124].

In cases presenting with fully developed classical motor features of PD, the clinical diagnosis may seem a straightforward exercise. However, early in the disease error rates for a clinical diagnosis of PD can be as high as 24% even in specialized centres. The most common misclassifications as PD in clinico-pathological series have been observed in cases of multiple system atrophy (MSA), progressive supranuclear palsy (PSP) and less frequently corticobasal degeneration (CBD), and in clinically based studies other common errors relate to essential tremor, drug-induced parkinsonism, and vascular parkinsonism[116]. Accuracy of a clinical diagnosis of PD can be improved significantly by the stringent use of standard clinical criteria, such as the UKPDSBB criteria, and a recent meta-analysis including 11 studies with pathologic examination as gold standard, revealed an overall diagnostic accuracy for the UKPDSBB of only 83%[125]. Diagnostic accuracy improves with a disease duration of 5 years or more[126], highlighting the need for diagnostic tests and biomarkers to enhance diagnostic confidence in early PD, or to even allow a definite diagnosis of prodromal PD.

[H2] Diagnostic tests and biomarkers

[H3] Imaging

Visualisation of striatal dopamine depletion in PD using 18F-labeled L-Dopa (fig 6) and PET was a breakthrough in molecular neuroimaging in 1985[127,128]. Since then, the field of neuroimaging has seen dramatic advances that are becoming increasingly relevant to PD[129-143]). 123I]N-w-fluoropropyl-2 β -carbomethoxy-3 β -(4-iodophenyl)nortropane ([123I]FP-CIT; ioflupane; DaTSan®), for example, is approved for clinical routine and can be used to differentiate between PD and clinical mimics that are not associated with presynaptic nigrostriatal terminal dysfunction[129,133,137]. Structural magnetic resonance imaging (MRI) helps to identify symptomatic parkinsonism[143] and a variety of MR techniques can reveal specific changes in the basal ganglia and infratentorial structures in atypical parkinsonism.

Advanced MRI techniques and post-processing procedures including diffusion weighted imaging, volumetric imaging, automated subcortical volume segmentation and multimodal imaging are being explored to enhance diagnostic accuracy for PD versus other types of degenerative parkinsonism[129,132,133,135,136,140,143]. Myocardial sympathetic denervation can be assessed with PET or SPECT using noradrenergic tracers and is common in PD, but not seen in patients with atypical parkinsonism or other PD mimics such as drug-induced parkinsonism or essential tremor[132-134]. An overview of imaging findings in PD is provided in table 2 and figure 6.

[H3] Genetics

The list of mutations causing monogenic forms of PD continues to grow as does the number of genes associated with complex phenotypes that include parkinsonism and have been assigned PARK loci (see table 1). Several other genes (including *GBA* on 1q22, *GCH1* on 14q22.1-2, *ADH1C* on 4q23; *TBP* on 6q27; *ATXN2* on 12q24.12; *MAPT* on 17q21.31; *GLUD2* on Xq24) have been identified which contribute to an increased risk for the sporadic form of the disease, of which the most prevalent and important are heterozygous mutations in the *GBA* gene. Large meta-analyses of datasets from GWAS have identified and confirmed many more common low-risk susceptibility variants in other loci in PD, that account for additional heritability, each potentially acting in a small but additive fashion[24,144,145]. In clinical practice, however, genetic forms only account for a small percentage (5-10%) of sporadic PD cases and as of yet genetic testing is not part of the routine diagnostic process, except in patients where there is a specific suspicion for a possible genetic cause (e.g. suggestive family history, early-onset – which is typical for several recessive genes - or specific clinical features like dystonia as a presenting symptom). Overall, the implications of genetic testing in clinical routine are limited by reduced penetrance and variable expressivity, and there is currently no impact of genetic findings on practical treatment decisions. This may well change in future as data from prospective studies on the prognostic implication of genetic mutations emerge and specific therapeutic targets in carriers of PD-associated mutations are being pursued. Examples for this are the recently reported increased risk for dementia in PD subjects carrying 'neuronopathic' GBA mutations[49,50] or development programmes for inhibitors of LRRK2.

[H3] CSF, blood and gut microbiota

Although several studies have assessed a variety of proteins in the CSF of PD patients versus controls (table 3) sensitivities and specificities for PD have been suboptimal and there is currently no clinically useful CSF diagnostic PD biomarker[128,146]. This is also true for blood biomarkers, although a recent study that found 11 plasma proteins (including apolipoprotein A1 (APOA1) to be associated with age of PD onset[147]. Interestingly, lower plasma APOA1 levels were associated with greater motor severity in early-stage, drug-naïve PD patients in several different PD-cohorts[148]. Increased levels of serum or plasma uric acid have also been found to associate with decreased PD risk and a meta-analysis calculated a pooled rate ratio of 0.80 for PD in people with one standard deviation increase in uric acid[149].

Alterations of gut microbiota have only recently moved into the focus of PD research with several studies showing differences in the gut microbiome composition in PD as compared to controls (table 3)[150]

The application of "-omics" techniques – such as proteomics, metabolomics and transcriptomics – are powerful tools capable of mass analyses to identify small changes in protein, metabolites or RNA profiles in fluids or even tissue from healthy and diseased individuals, and they have been started to be used in PD[127]. The recent initiation of large multi-site consortia for PD biomarker development such as the Michael J. Fox Foundation's Parkinson's Progression Marker Initiative (PPMI) and the National Institutes of Neurological Disease and Stroke PD Biomarkers Program holds promise for the discovery of new powerful biomarkers[146].

[H3] Tissue Biopsies

Recent studies have tried to detect α-synuclein-related pathology in the peripheral autonomic nervous system using skin punch biopsies, biopsies of the salivary glands as well as gastrointestinal biopsies with the ultimate goal to define diagnostic markers for the earliest stages of PD[66]. Although results have been inconsistent between studies, there is converging evidence for a diagnostic role of immunostaining for phosphorylated α-synuclein in autonomic ganglia and fibers in skin, colonic or submandibular gland biopsies. For example, two studies found increased aggregation of α-synuclein and fibre loss in autonomic sudomotor and pilomotor fibres in skin punch biopsies, separating PD patients from controls with a high diagnostic accuracy[151,152]. Intriguingly , recent studies have also found immunostaining for phosphorylated α-synuclein in colonic and submandibular gland biopsies in subjects with iRBD, suggesting possible usefulness of biopsies to detect prodromal PD (see below)[153,154].

[H2] Screening and prevention

Today neuronal dysfunction in PD is believed to start long before the defining motor features have become apparent[155]. Affected subjects may either be asymptomatic ("preclinical PD") or present with a variety of non-motor symptoms and/or subtle motor signs that do not meet the current diagnostic criteria for PD ("prodromal PD"). In an attempt to define at-risk subjects or prodromal disease stages current research efforts have focused on factors, that have been associated with later diagnosis of PD in epidemiological studies (Box 1)[146,156]. Subtle motor abnormalities ('mild parkinsonian signs) such as reduced arm swing, changes in walking patterns, stiffness, tremor, or changes of fine motor skills may be

found in as many as 40% of the elderly population where they have been associated with an increased risk for incident PD or with other PD risk markers[66]. In addition, several studies have demonstrated that subtle motor abnormalities can be detected in PD subjects already considerably before the eventual diagnosis, and more sensitive digital tools to identify these early motor features are being tested.[157]

Non-motor symptoms appear to long antedate the onset of classical motor symptoms in PD subjects (figure 5)[66,156,158] . Idiopathic REM-sleep behaviour disorders, in particular, carries a high risk for the development of PD or other α-synucleinopathies[156]. Recent prospective studies in large RBD cohorts have found conversion rates to PD, dementia or MSA between 15% and 40% over 2 to 5 years and up 90% with longer follow-up beyond 10 years, but the need for polysomnographic confirmation limits the utility of RBD for screening at the population level.

Hyposmia, on the other hand, is an established, albeit less specific risk factor for PD which is easy to screen at relatively low cost and has a relatively high prevalence in the general population [66]. A two-step approach using smell testing as a primary screen and dopamine transporter scan imaging as a secondary screen in hyposmic individuals is being used as a screening approach in the population-based PARS study where hyposmia when combined with older age and constipation was associated with DAT deficits in >40% of such individuals[159]. In a small cohort of subjects with iRBD the presence of hyposmia yielded a predictive value for conversion to PD or PD dementia of more than 60% over 5 years[160] which has obvious implications for the calculation of sample sizes for future 'neuropreventive' trials targeting prodromal PD.

The International Parkinson and Movement Disorder Society (MDS) have recently published research diagnostic criteria for prodromal PD which are based on epidemiological data about the effects of a large number of risk and prodromal markers[65]. Their predictive validity and therefore usefulness for selecting populations for 'disease-prevention' trials still awaits prospective testing, but a recent population-based study has provided first evidence to this effect[161].

[H1] Management

[H2] Dopaminergic pharmacological targets

Loss of dopaminergic neurons in the substantia nigra pars compacta leading to striatal DA depletion is the core mechanism underlying the cardinal motor features of PD. Substituting striatal dopamine loss via the systemic administration of the dopamine-precursor amino acid L-Dopa represented a revolutionary breakthrough in the treatment of PD more than 50 years ago. Since then, important advances in the understanding of the pharmacological players regulating nigrostriatal dopaminergic transmission have revealed multiple additional targets for dopaminergic therapies in PD. These can be broadly classified into interventions with primarily presynaptic or postsynaptic activity (fig.7).

[H3] L-Dopa

L-dopa has remained the gold standard for symptomatic efficacy of any antiparkinsonian drug and over time practically all PD patients will require treatment with this agent[162,163]. Its use is, however, complicated by the evolution of motor complications including motor response oscillations and drug-induced dyskinesias (Box 2). The mechanisms underlying these phenomena, in particular those responsible for the development of dyskinesias with

chronic L-dopa replacement, are still incompletely understood. Both pre- and postsynaptic mechanisms are involved, eventually leading to unphysiological pulsatile striatal dopamine receptor stimulation and giving rise to a variety of maladaptive neuronal responses (fig. 7)[164,165]. The key cause is discontinuous drug delivery due to the short half-life of L-dopa and variability in its gastrointestinal absorption and blood-brain-barrier transport (Box 2[166]). Novel sustained-release formulations of L-dopa as well as continuous delivery (either intestinally via PEG-J tubes or subcutaneously via mini-pumps) have been or are being developed to address this problem[166]. Clinical observations of reductions of pre-existing dyskinesias with intestinal gel infusions of L-dopa indeed support the value of the concept of continuous dopaminergic receptor stimulation as a means to prevent the evolution of drug-induced dyskinesias[166,167].

[H3] COMT-Inhibitors

Current L-dopa preparations include inhibitors of aromatic amino acid-decarboxyalse (AADC; carbidopa or benserazide) in order to prevent peripheral metabolism to dopamine and enhance bioavailability. As a consequence, the peripheral metabolism of L-Dopa is shifted towards the activity of a secondary metabolic pathway involving ortho-methylation of L-Dopa via catechol-o-methyl-transferase (COMT). Inhibiting this enzyme in the periphery will further enhance bioavailability and half-life of levodopa which is of particular benefit in patients who have developed motor fluctuations of the wearing-off type[168]. Extending the duration of effect of individual levodopa doses via COMT-inhibitors has become a first-line treatment in these subjects and currently three preparations are available for clinical use[169,170].

[H3] MAO-B Inhibitors

Oxidation via monoamine oxidase type B (MAO-B) in glial cells is a major clearance mechanism for synaptically released dopamine, next to presynaptic reuptake via the dopamine transporter (DAT)[171] (see fig. 7). Inhibition of this enzyme prolongs and increases synaptic dopamine concentrations and symptomatic efficacy of MAO-B inhibition using the selective inhibitor selegiline as an adjunct to Levodopa was shown already in the 1970s[172]. More recent studies have established the antiparkinsonian efficacy of monotherapy with selegiline and the newer MAO-B inhibitor rasagiline and the latter has also been found efficacious when added to levodopa in patients with motor fluctuations[169] . While both, selegiline and rasagiline are irreversible (“suicide”) inhibitors of the MAO-B-enzyme, the most recent marketed agent with MAO-B acitivity, safinamide, acts as a reversible MAO-B inhibitor[173].

[H3] DA-Agonists

The actions of dopamine on striatal medium spiny neurons are mediated via two classes of dopamine receptors (D1 and D2 receptors; see fig 7). Physiological activity of dopamine at D1 receptors results in activation of the direct striatopallidal projection while stimulation of D2 receptors inhibits firing in the indirect striatopallidal pathway. The net result of striatal dopamine release is motor facilitation via enhanced glutamatergic transmission from the thalamic motor nuclei to the prefrontal motor cortex (see above). Dopaminomimetics with direct activity to dopamine receptors (DA receptor agonist) were first introduced into PD therapy in the 1970s with the ergot alkoloid bromocriptine and have since become an important medical therapy for PD motor symptoms[169,174]. Initial members of this family of drugs had ergoline structure and activity at 5-HTB2 receptors which became associated with

pleuropulmonary and cardiac valvular fibrosis introducing important safety concern. Currently used agents are all non-ergoline drugs and devoid of this activity. An important advantage of dopamine agonists is their longer half-life as compared to L-Dopa which makes them attractive candidates as adjunct therapies in patients with motor fluctuations[169,175]. In addition, non-ergoline agonist rotigotine is available as a transdermal patch formulation affording continuous drug elivery. Overall, DA agonists are believed to induce less pulsatile striatal dopamine receptor stimulation as compared to L-Dopa and this is taken as an explanation for the markedly reduced risk to induce motor complications when dopamine agonists are used as initial monotherapy in PD[174,175]. Drawbacks include their reduced overall effect size as compared to L-Dopa and their potential to induce drowsiness and impulse dyscontrol – the latter being possibly associated with their preferential activity at D3 receptors located in the ventral striatum causing excessive stimulation of brain reward systems[176]. Apomorphine stands out among the other dopamine agonists in terms of combined activity at both, D1 and D2 receptors and equipotency to levodopa[177]. Continuous subcutaneous apomorphine infusions not only smoothen out motor response fluctuations but have also been associated with reductions of pre-existing levodopa-induced dyskinesias[178]. Currently new apomorphine formulations, for sublingual use, are in clinical development[179].

[H2] Non-Dopaminergic Pharmacological Targets

Despite the remarkable impact of dopaminergic therapy on the symptoms of PD, there is a clear need for therapies that target other pharmacological systems. The symptom categories that need to be addressed by such treatments include the complications of levodopa therapy like motor fluctuations and levodopa-induced dyskinesia (LID) as well as levodopa-resistant ("non-dopaminergic") motor features including treatment-resistant tremor, freezing of gait (FOG), postural instability and falls, swallowing and speech disturbances. Currently, the only available and effective pharmacological treatment for L ID is amantadine, which is thought to work as an NMDA antagonist[169,174,175]. Table 4 provides a summary of non-dopaminergic pharmacologic treatments that are used or are under development to address various motor problems in PD.

In some patients, selected non-motor complaints (e.g. pain, anxiety, panic, depression, restlessness) can fluctuate in response to dopaminergic therapy and these “non-motor fluctuations” can be equally or more disabling than the motor symptoms[121,180]. Many non-motor symptoms do not respond to dopamine replacement therapy and some are indeed aggravated or precipitated by this treatment[121]. Anatomic targets for these problems include afferent, efferent and intrinsic basal ganglia connections, a variety of brainstem-originating projections as well as intrinsic cortical connections and finally, a number of targets outside the central nervous system (for example, in the peripheral autonomic nervous system)[181]. Non-dopaminergic neurotransmitter and neuromodulatory systems in these various regions that have been implicated in the symptoms of PD include glutamatergic, adenosinergic, noradrenergic, serotonerigic, GABAergic, opioid, cholinergic and histaminergic pathways[182].

Cognitive dysfunction, depression and autonomic failure are among the most prevalent and troublesome non-motor issues in PD. Cholinesterase inhibitors can have striking beneficial effects on the cognitive disturbances of PD patients with dementia, an effect possibly related to the significant loss of cholinergic projections from the nucleus basalis of Meynert in PD[183,184]. The most effective therapy for psychotic symptoms in PD is clozapine[183 184]. All other available atypical neuroleptics, apart from quetiapine, worsen parkinsonism probably

by blocking striatal D2 receptors. The exact mechanism of action of clozapine in Parkinson's psychosis is uncertain; a serotonergic effect is strongly supported by the recent positive results using the 5-HT2A inverse agonist pimavanserin[185]. It is not known whether depression in PD has the same anatomic and pathogenic bases as depression in the general population[186]. In fact, like depression in other circumstances, it is likely that depression in PD is not a uniform, homogeneous disorder. Although patients may respond to all types of antidepressant medications there is limited evidence that tricyclic antidepressants may be more effective than SSRIs, suggesting a greater role for noradrenaline systems, although this remains to be firmly established[183]. Finally, autonomic dysfunction is extremely common particularly in late-stage PD and pharmacological therapies are largely directed at peripheral autonomic nervous system targets. They include the mineralcorticoid fludrocortisone and adrenergic agents like midodrine, etelifrine or the noradrenaline pre-cursor droxidopa to treat orthostatic hypotension, anti-muscarinics like oxybutinine, tolterodine or trospium chloride for urinary urgency or incontinence and pro-kinetic drugs like macrogol or lubriprostone to improve constipation[174,183,187].

[H2] Deep brain stimulation

Deep brain stimulation (DBS) is a surgical treatment for PD, which is based on the empirical observation, that high-frequency (100-200 Hz) electrical stimulation of specific brain targets can mimic the effect of a lesion without the need for destroying brain tissue. It is accomplished by implanting an electrode into the targeted brain area and connecting it to an internal pulse generator, which can be programmed telemetrically.

DBS has rapidly replaced ablative stereotactic surgery for movement disorders due to several advantages: (a) DBS does not require making a destructive lesion in the brain, (b) it can be performed bilaterally with relative safety in contrast to most lesioning procedures, (c) stimulation parameters can be adjusted postoperatively to improve efficacy, to reduce adverse effects and to adapt DBS to the course of disease, and (d) DBS is in principle reversible and does not preclude the use of possible future therapies in PD requiring integrity of the basal ganglia circuitry.

The breakthrough for DBS as a treatment for PD came in 1993 when new concepts of the basal ganglia circuitry prompted the team around the neurologist Pierre Pollack and the neurosurgeon Alim Benabid in Grenoble to implant the subthalamic nucleus (STN) (fig. 4b) as a novel target for chronic high-frequency neurostimulation[188]. Since then a multitude of clinical trials has confirmed the initial observation on the dramatic antiparkinsonian efficacy of STN-DBS which is now an established evidence-based therapy for motor fluctuations and dyskinesia in patients with advanced PD[169]. The stimulation-induced improvement is closely linked to the responsiveness of motor symptoms to dopaminergic treatment in a given patient. The only exception to this rule is drug-resistant tremor in a patient with otherwise good response to L-Dopa, where DBS can succesfully control tremor. In general, ideal candidates should suffer from idiopathic PD with an excellent levodopa response (as demonstrated by history and a pre-surgical L-Dopa test) but motor complications of long-term medical treatment[189]. Dementia, acute psychosis and major depression are exclusion criteria[189]. Patients with young-onset PD fulfill the inclusion criteria for DBS best and are overrepresented among the operated group. Older age is not necessarily an exclusion criterion for surgery, but levodopa-resistant symptoms are more often encountered in this group, surgical adverse events are more frequent, motor rehabilitation is slower and frailty

may limit the degree of functional restitution. Bilateral STN-DBS reduces the UPDRS II (activities of daily living) and III (motor) scores on average by 50-60% compared to the preoperative medical off-state. The levodopa equivalent daily drug dosage is reduced following surgery by an average of 60%. As a consequence, dyskinesias decrease by 60-70% and hypokinetic fluctuations are markedly reduced with a decrease of daily off-time by approximately 70%[190]. Several randomized controlled clinical trials have proven that DBS provides a better quality of life than best medical management in patients suffering from clinically relevant motor fluctuations and dyskinesia[190].

The internal globus pallidus is an alternative surgical target for the treatment of motor complications, but does usually not allow to reduce medication[191] . Randomised trials comparing the two targets have produced conflicting results of either similar motor benefits[192] or inferior long-term efficacy on motor function and motor fluctuations of Gpi- versus STN-DBS[193] .

DBS is a complex therapy requiring a high level of interdisciplinary expertise in the correct surgical placement of the electrode, postoperative programming and the adjustment of neurostimulation and drug therapy[189]. The most relevant adverse events related to DBS are intracranial bleedings and device complications (infections, lead misplacements, etc.), which account for a permanent morbidity of less than 1-3%. The mortality of DBS is below 0.5%[194]. Therefore, the benefit-risk profile of DBS is usually considered to be favorable, in particular with respect to the large gains in quality of life compared to best medical management observed in clinical trials. Psychiatric sequelae of DBS (e.g. apathy, depression, impulsiveness or mania) are not uncommon and result from a complex interplay between disease related psychiatric symptoms, dopaminergic imbalance due to the profound medication changes and stimulation-induced effects on limbic basal ganglia circuits[195]. Better devices allowing a finer control over the spatial distribution of current around the electrode, closed-loop neurostimulation systems autoadjusting parameters based on physiomarkers and computer assisted expert systems for surgical planning and postoperative programming are now becoming available and may help to make the procedure less dependent of expert knowledge and provide more consistent outcomes across centers[196].

[H2] Exercise-based Treatment

Most patients with PD have to cope with residual motor disabilities affecting gait and mobility, postural control and balance, as well as speech and swallowing function, which are often poorly responsive to drugs and mostly unresponsive to DBS. In addition to historic use, a steadily increasing number of trials document the effects of a variety of exercise-based strategies on classical motor outcomes like the UPDRS, but also specific parameters like gait speed, balance control, freezing, muscle strength and speech or global measures like quality of life[169,197,198]. These developments are paralleled by a new research evidence for neuroplastic and potential neuroprotective effects of activity enhancing approaches in experimental models[199] as well as epidemiological evidence for PD risk modulation by physical activity[200,201].

[H1] Quality of life

Whilst motor symptoms have been in the foreground of clinical approaches to PD the introduction of patient-reported health-related quality of life (QoL) measures led to the recognition that the multitude of motor and non-motor features of PD has a wider impact on patients' overall health than just motor impairment[121]. It is clear that QoL deteriorates with advancing disease and worsening motor disability[202], but in optimally treated patients with PD non-motor features, in particular autonomic, cognitive and psychiatric aspects are of greater importance to patients QoL[203]. Many non-motor symptoms are under-reported and under-recognized, and only in the last years have treatment trials for specific non-motor symptoms been conducted[183,204]. The most consistently reported non-motor feature of PD associated with poorer QoL is depressive mood, including subsyndromal depression[186]. Most studies have excluded patients with dementia from QoL studies, but evidence is emerging that the cognitive impairment is also a significant contributor to patients' QoL even at early stages[205]. The range of other non-motor symptoms seen in PD, including constipation, urinary urgency, insomnia, fatigue, pain, other neuropsychiatric presentations and sexual dysfunction, contribute to lower QoL scores particularly in advanced disease, and are potentially treatable[206].

Clinical trials on the effect of treatments on both motor as well as non-motor symptoms in PD now frequently include QoL measures. QoL measures have been used to combine assessment of efficacy and tolerability of treatments, and compare overall impact of treatments, on patients' overall QoL, rather than on specific disease aspects alone. Examples of treatments that have demonstrated improvement of QoL[207] in patients with PD include antiparkinsonian medications or DBS surgery[162] exercise[198], multidisciplinary intervention, as well treatments of non-motor symptoms and comorbid conditions[204].

Carer wellbeing in PD is also affected by the challenges of caring for a person with both physical impairment as well as mental health issues such as depression, psychosis and cognitive impairment. The most important contributing factors to carer burden include dementia, falls and non-motor symptoms[208], and patient QoL and mood are closely related to carer burden[209]. Currently, no interventions are validated to improve carer burden, although a number of trials to improve carer burden are under way.

[H1] Outlook

[H2] Challenges

A major PD research challenge is that environmental (e.g. pollutants) and lifestyle (e.g. diet, exercise, smoking) factors likely contribute to lifetime risk for PD, in part by affecting the epigenome in the nervous system, and these factors are difficult to accurately measure in large cohorts over the decades that they play a role. A second challenge is that PD probably is a multifactorial disease. Thus, both upstream pathogenic molecular mechanisms and some of their downstream effects likely differ between patients, even if the final outcomes (α-synuclein aggregation and nigral neurodegeneration) are shared. A third challenge is that PD symptomatology, clinical course and neuropathology varies between patients, which supports the idea that several forms of molecular pathogenesis coexist within the diagnostic realms of PD. A fourth challenge is that development of PD might conceivably require the simultaneous activation of more than one pathogenic pathway, and that certain cellular defense mechanisms fail concomitantly. Finally, superimposed on the complex interplay of multiple triggers and failing defenses is the process of normal cellular ageing. The greatest risk factor for PD is increasing age and, possibly, the same molecular perturbations that are handled gracefully by a young neuron have catastrophic consequences in an aged counterpart.

All this makes the development and testing in clinical trials of putative disease-modifying interventions extremely complex and risks to fail the test of controlled trials are high.

[H2] Predictive factors

Although the future of PD research is thus faced with a multitude of challenges there is now also for the first time a realistic possibility to define populations at risk or subjects in the earliest stages of PD. Based on multiple prospective population-based studies a variety of factors that are associated with an increased risk to develop PD have been identified. These include behavioral aspects like non-smoking or non-use of caffeine, physiological changes like hyposmia, constipation or subtle motor abnormalities and – most strongly of all – the presence of RBD. Genetic, proteomic, metabolomic and tissue biomarkers are also being increasingly characterized and neuro-imaging may have an important future role in providing information on PD risk. Current prospective cohort studies are trying to assess the sensitivities, specificities and predictive value of various risk markers for PD. Such data will be critical to define cohorts for future disease modification studies in at risk populations. The recently proposed MDS research criteria for prodromal PD have already been tested retrospectively in one population-based study and hold promise as a first operational step towards future preventive studies[161].

[H2] Experimental therapies

Two highly experimental techniques focused on achieving structural or neurochemical brain repair in PD have generated great interest during recent decades, namely gene therapy and cell transplantation. Although progress towards successful clinical translation has not been rapid in either case, both remain conceptually important approaches that, if successful, can have great clinical impact. In addition, recent advances in our understanding of the molecular pathogenesis of PD (see section 3.2) have revealed novel therapeutic targets for disease-modifying pharmacological therapies.

[H3] Gene therapy

For gene therapy, two main strategies exist: viral-vector mediated expression of growth factors or neurotransmitter-synthesizing enzymes. A vast body of experimental evidence suggests that members of the glial cell-line derived neurotrophic factor (GDNF) family protect nigral dopamine neurons from death and promote regeneration of their axons following damage[210]. A small open label trial with GDNF injections into the putamen in PD generated optimism by suggesting that GDNF might reduce PD symptoms[211] , but a larger placebo-controlled clinical trial with injections of smaller doses of GDNF failed to show clinical benefit[212]. This disappointing outcome of the controlled GDNF trial did not deter the company Ceregene Inc. from testing gene therapy that induced expression of neurturin, a less potent member of the GDNF family of growth factors that had demonstrated efficacy in PD animal models[213]. In a series of trials targeting putamen or both putamen and substantia nigra, it was found that adeno-associated virus (AAV)-mediated expression of neurturin was safe[214,215], but again neither approach stood the test of randomized clinical trials[216,217]. Post-mortem findings suggested that the neurturin was expressed only in relatively few cells surrounding the injection tracts limiting its neurorestorative potential and it has been suggested to target earlier stages of PD, when more of the nigrostriatal axons still remain functional[218,219]. An ongoing safety trial (predicted to close in 2018) is testing the effects of AAV-mediated expression of GDNF in PD, and with improved vector technology and better understanding of the effects of growth factors, it is likely that there will be additional attempts at inducing neurorestoration in PD in the future[218,219].

Clinical trials are also underway using viral vector-mediated expression of key enzymes in the dopamine-synthesis pathway. Thus, in different studies lentiviral (LV)- and AAV-vectors expressing tyrosine hydroxylase (TH) with cofactors and amino acid decarboxylase (AADC) have been injected into the striatum, with initial safety reports already published[220,221]. The strategy is to genetically modify cells in the striatum so that they can produce and release dopamine locally, either from tyrosine or from peripherally administered L-DOPA or dopamine. Animal studies have demonstrated that this approach is feasible and that it might not just provide relief of dopamine-dependent motor symptoms, but by providing constant dopamine receptor stimulation it could also reduce the risk of motor fluctuations developing later on[222,223]. Another approach has targeted the subthalamic nucleus with AAV2-Vector mediated delivery GAD to induce GABAergic inhibition of STN firing with promising results from a sham-surgery controlled phase 2 trial[224] .

[H3] Fetal cell transplantation

In the 1990's, cell transplantation was considered a promising approach to brain repair in PD. Open label trials suggested that immature dopamine neurons obtained from aborted embryos/fetuses not only could restore striatal dopamine transmission and connectivity (as evidenced by in vivo positron emission tomography and morphological findings upon autopsy), but also could reduce the motor symptoms[225,226]. However, the publication of two NIH-sponsored, double blind placebo controlled trials in 2001 and 2003 brought the clinical programs to a standstill[227,228]. Not only was there no evidence of clinical benefit in these trials, but it was also reported that some patients developed uncontrollable graft-induced dyskinesias (GID)[227,228], which was also confirmed in a retrospective analysis of patients in the open label trials[229]. While the clinical trials were halted, laboratory research into the mechanisms underlying GIDs started at the same time as the modern era of research into regenerative stem cell therapies was born. Based on animal experiments and clinical

observations, today it is believed that GIDs are unlikely to develop if the number of serotonergic neurons included in the dissected graft tissue is minimized[230] and if the selected patients do not already exhibit L-DOPA-induced dyskinesias prior to surgery[231]. In 2008, concerns were raised about the future of the cell transplantation approach when it was reported that Lewy pathology appears inside grafted neurons over one decade after surgery. Today the consensus is that there are progressive signs of degeneration (loss of DAT and TH, as well as appearance of Lewy pathology) in grafted neurons[232], but that it takes well over a decade before these pathological changes might impair the function of the transplants. In open label setting, there is quantitative evidence of beneficial graft effects in at least two cases up to 15-18 years after surgery[233]. In 2015, an open label study with fetal dopamine neuron implants was initiated by the European Union funded team TRANSEURO, and this group plans to have operated on 20 patients, all at a relatively early stage of disease, before the end of 2017[234,235]. This trial will help to clarify if GIDs can be avoided and will also help lay the foundations for future trials that use stem cell-derived neurons as the source of transplantable dopamine neurons.

[H3] Stem cells as donor tissue

Over the past 10-15 years stem cell therapy in experimental PD has developed dramatically[234]. Today it is possible to generate dopamine neurons with midbrain characteristics from two forms of human pluripotent stem cells, namely human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC)[234]. Recent studies have shown that they survive grafting to experimental animals, grow axons that innervate the brain and support functional recovery from lesion-induced deficits[234]. Current research is focused on resolving issues related to scaling up production of cells, guaranteeing safety and meeting the increasing regulatory demands placed on biological products[234]. While several commercial entities already advertise different types of stem cell therapies for PD, the underlying scientific rationale for using the proposed cell type is frequently not strong[236]. The development of a stem cell based trials should follow the guidelines set forth by the International Society for Stem Cell Research[237]. One can expect that such clinical cell transplantation trials in PD that use stem cell-derived products which are well validated scientifically will commence 2-3 years from now. Progress in this area is stimulated by the existence of an international consortium of scientists called G-Force-PD, which is devoted to clinical translation of stem cell therapy in PD and holds regular meetings where technical advances and protocols are shared[234].

[H3] Novel targets for disease modification

The growing evidence for oligomerisation and fibrillar aggregation of pathological α-synuclein species and their possible cell-to-cell transmission as a key event in the molecular pathogenesis of PD has moved multimerisation and extra-and intracellular handling of this protein into focus as targets for novel therapies. Two immunological approaches are currently in active clinical development. First, active immunisation with a novel vaccine containing short peptides homologuous to α-synuclein conjugated to a carrier (AFFITOPE ^{R}) induced the formation of antibodies specifically directed to the C-terminus of human α-synuclein, cleared α-synuclein aggregates and reduced neuropatholgy in a transgenic PD mouse model[238]. A phase 1/2 safety trial in 28 subjects with PD also provided evidence for induction antibody formation against α-synuclein without safety concerns, as well as immunological efficacy of booster vaccinations up to 3 years after initial immunisation[239]. A

placebo-controlled phase 2 trial of AFF03, a second AFFITOPE technology vaccine against α-synuclein is currently ongoing.

A second approach is passive immunisation via monoclonal antibodies antibodies against α-synuclein and to date 3 candidate compounds have reached early phases of clinical development phase[240]. Following the first positive phase 2 results with an amyloid-beta monoclonal antibody in Alzheimer's Disease[241], this field will almost certainly gain momentum over the next 5 years also in PD.

Other α-synuclein targeting approaches focus on extracellular synuclein binding sites, inhibitors of α-synuclein aggregation or enhancers of α-synuclein clearance via the LAS[64,242,243], but have not yet progressed into clinical development (see fig. 8).

Other experimental approaches, however, pursue different targets including the GLP-1R agonist exenatide[244], the urate precursor inosine[245] , the GBA chaperone ambroxole[246] or the Ca^{2+} channel antagonist isradipine[247] and some of these are currently being tested in clinical PD trials.

[H2] Clinical trials.

To date all clinical disease-modification trials in PD have been performed in subjects with early PD as defined by the presence of classical motor features and they have all failed to show unequivocal positive effects. Studying interventions in the prodromal or 'pre-clinical' phase of disease should offer greater promise of success assuming less advanced pathology and greater potential to intervene at critical points of molecular pathogenesis. These may have greater likelihood of success if patients recruited into clinical trials can be shown to share some form of biomarker that relates to the therapeutically targetted pathogenic mechanism.

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Figure legends

FIGURE 1

Title: Incidence and prevalence of PD. a: Prevalance per 1 Million; b: Incidence rate per 100,000 (derived from 2 recent meta-analyses which used crude rates without adjustments for demographic differences or methodological differences between studies [248,249])

FIGURE 2

Title: The main diagnostic neuropathologies for PD.

A Macroscopically, PD is defined by depigmentation of the midbrain substantia nigra (SN), as observed in these transverse sections of the midbrain, upon immunohistochemical staining tyrosine hydroxylase, the rate limiting enzyme for the synthesis of dopamine. Selective loss of the ventrolateral parts of the SN with sparing of the more medial and dorsal regions is evident. 3N=3rd nerve fibres, cp=cerebral peduncle, R=red nucleus.

B-C Haematoxylin and eosin stainin of the ventrolateral region of the SN showing a normal distribution of pigmented neurons in healthy control (B) and diagnostically significant moderate (C) or severe (D) pigmented cell loss in PD.

E-G Immunohistochemal staining of α-synuclein shows the round, intracytoplasmic Lewy bodies (arrowhead in E), more diffuse, granular deposits of α-synuclein (E, F), deposits in neuronal cell processes (F), extracellular dot-like α-synuclein structures (F) and α-synuclein spheroids in axons (G).

H The theorized progression of α-synuclein aggregation in PD without Alzheimer pathology. α-Synuclein inclusions occur in cholinergic and monoaminergic lower brainstem neurons in asymptomatic cases (Braak Stage 1/2), infiltrate similar neurons in the midbrain and basal forebrain in those with the motor symptoms of PD (Braak Stage 3/4), and then are found later in limbic and neocortical brain regions with disease progression (Braak Stage 5/6)[250].

FIGURE 3

Title: Molecular mechanisms involved in PD

FIGURE 4

Title: Motor cortex circuitry activity changes in PD

The motor circuit consists of corticostriatal projections from primary motor cortex, SMA, cingulate motor cortex and premotor cortex terminating upon dendrites of striatal medium spiny neurons. The hyperdirect pathway has direct glutamatergic connectivity from the motor cortex to the subthalamic nucleus (a).

The internal globus pallidus (GPi) and the substantia nigra pars reticulate (SNr) are the two major output nuclei of the basal ganglia and project to the brainstem and ventrolateral thalamus. The striatal projections to these output nuclei are divided into a “direct” and an “indirect” pathway. The direct pathway is a monosynaptic connection between predominantly D1-receptor expressing medium spiny neurons and GABAergic SNr and GPi neurons. The “indirect” pathway originates from D2 expressing medium spiny neurons, which project to the external globus pallidus, and reaches the internal globus pallidus via the subthalamic nucleus as a glutamatergic relay. Through these two reins the striatal dopaminergic tone regulates the GABAergic output activity of the basal ganglia.

b: Changes associated with Parkinsonism: Nigrostriatal dopamine deficiency has opposing effects on the direct and indirect pathway While D1-mediated direct pathway activity becomes reduced, D2-mediated indirect pathway activity increases, resulting in the net effect of a strong increase in the firing rate of GABAergic basal ganglia output neurons, which over inhibit downstream thalamo-cortical and brainstem areas
MC= motor cortex

FIGURE 5:
Title: Clinical symptoms and PD progression
Diagnosis of PD occurs with the onset of motor symptoms (early stage PD) typically in the late fifties, but can be preceded by a prodromal phase of years or even decades, which is characterised by specific non-motor symptoms (prodromal PD). Non-motor symptoms become increasingly prevalent and obvious over the course of the illness, but can present to a variable degree throughout all stages of PD. Progressive disability from PD is driven by the combination of these non-motor problems with increasing severity of cardinal motor features, the development of levodopa-induced motor complications (mid stage PD) and the evolution of poorly levodopa responsive motor disabilities like postural instability, gait problems including freezing and dysphagia (late stage PD). Aging itself is likely to have significant impact on the development in key disability milestones (i.e. frequent falls, visual hallucinations, dementia and need for institutionalisation) in PD, which are reached at a very similar age of between 70 and 75 years about 3 to 6 years before death.
Abbreviations: EDS=excessive daytime sleepiness, MCI=mild cognitive impairment, PIGD=postural instability and gait disorder, RBD=REM sleep behaviour disorder.

FIGURE 6
Title: Imaging methods used to study Parkinson's disease
Part 1) Various approaches to the assessment of the integrity of the dopaminergic nerve terminal using different radiotracers with PET and single-photon emission CT: presynaptic dopamine activity (dopamine transporter, vesicle transporter and dopamine storage) and postsynaptic dopaminergic system (D2/D3 receptors). Part 2) ^{18}F-DOPA-PET images of a healthy control individual and a patient with predominantly left-side affected early PD. In the healthy control, there is normal tracer uptake bilaterally in putamen and caudate nuclei with the typical comma-shaped structure due to the putamen, while in the PD patient there is asymmetric uptake with more marked reduction in the right compared to the left putamen. Part 3) Myocardial sympathetic innervation can be studied with MIBG SPECT and MHED or ^{18}F-dopamine PET. Planar cardiac delayed MIBG imaging in a control and an early PD. There is markedly reduced MIBG uptake in the heart (H) in the patient with PD compared to the mediastinum (M). Part 4) A) shows a SWI image of a healthy control, demonstrating the magnified dorsolateral nigral hyperintensity within the right substantia nigra. Yellow arrows mark the DNH in the survey as well as in the magnified illustration. B) shows SWI images demonstrating the DNH in a healthy control, its absence in a patient with PD. Part 5) Ultrasound images of the substantia nigra. A) show a plane of the mesencephalic brainstem. B) demonstrates typical examples of transcranial ultrasound appearances (axial scanning plane) in a patient with PD and a HC. The outer line marks the midbrain area, the inner line marks the ipsilateral echogenic area at the anatomical site of the SN on the side of insonation. Part 6) Neuromelanin-sensitive MRI of the SN pars compacta. Compared with the healthy control, a patient with early PD shows reduced signal intensity in the lateral part of

the substantia nigra pars compacta (black circles and arrows). Large white oval and circle show no changes in the periaqueductal grey matter.

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Part 4) reprinted from Reiter, E. et al. Mov Disord. 2015;30:1068-76 (figure 2), with permission from Wiley; part 5) A) reprinted from Godau et al. Mov Disord 2012 27:634-43 (figure 4), with permission from Wiley; B) reprinted from Schmidauer et al. Annals of Neurology 2005;58:630-634 (figure 2), with permission from Wiley; part 6) reprinted from Ohtsuka et al. Neurosci Lett. 2013 541:93-8 (figure), with permission from Elsevier.

Abbreviations: ß-CIT=2beta-carbomethoxy-3beta-(4-iodophenyl)tropane . CFT= 2β-carbomethoxy-3β-(4-fluorophenyl)tropane. DTBZ=dihydrotetrabenazine. FP-CIT=N-w-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane . IBZM=iodobenzamide. MIBG=metaiodobenzylguanidine. MHED = ^{11}C-metahydroxyephedrine. MP=d-threo-methylphenidate. TRODAT=2[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3,2,1]-oct-2-yl]-methyl](2-mercaptoethyl) amino]ethyl]amino]ethane-thiolato(3-)-N2,N2',S2,S2]oxo-[1R-(exo-exo). M=ROI regarding MIBG uptake in the mediastinum. H=ROI regarding MIBG uptake in the heart. GP/Put=globus pallidus/putamen. PMC=premotor cortex. DNH=dorsolateral nigral hyperintensity; N=dorsolateral nigral hyperintensity (in figure). SN=substantia nigra. RN=red nucleus. SWI= susceptibility-weighted imaging. PD=Parkinson disease. HC=healthy control. ROI=Regions of interest

FIGURE 7
Title: Dopaminergic drug targets to the treat the motor symptoms of PD
Presynaptic targets include levodopa substitution combined with peripherally active inhibitors of DOPA-decarboxylase (DDC) or C-O-methyl-transferase (COMT). MAO-B inhibitors enhance synaptic availability of dopamine (both endogenous and exogenous) while dopamine agonists act both synpatically.
Abbreviations: TH = tyrosine hydroxylase; DDC = DOPA-decarboxylase, DAT = dopamine transporter, DOPA = deoxyphenyylacetic acid, 3-O-MDOPA = 3-O-methyl-DOPA

Box 1

Overview of large-scaled studies exploring biomarkers for PD

Biomarker studies in manifest PD: PPMI, BioFIND, PDBP, OPDC, DeNoPa, COPPADIS-2015, ICICLE-PD, ParkWest

Biomarker studies to define Prodromal PD: PRIPS, PPMI (prodromal and genetic cohorts), OPDC (at-risk cohort), PARS, PREDICT-PD

Abbreviations: BioFIND = Fox Investigation for New Discovery of Biomarkers in Parkinson's Disease; COPPADIS-2015 = COhort of Patients with PArkinson's DIsease in Spain, 2015); DeNoPa = De Novo Parkinson study; ICICLE-PD = Incidence of Cognitive Impairment in Cohorts with Longitudinal Evaluation – Parkinson's Disease study; OPDC = Oxford Parkinson Disease Centre (OPDC) Discovery Cohort; ParkWest = Norwegian ParkWest Study; PARS = Parkinson Associated Risk Syndrome Study; PDBP = NINDS Parkinson's disease biomarkers program; PPMI = Parkinson's Progression Markers Initiative; PRIPS = Prospective validation of Risk factors for the development of Parkinson Syndromes study;

Box 2

L-Dopa induced motor complications

Chronic exposure of PD patients to L-Dopa is associated with the development of motor complications in about 30 % of patients after 2-3 years and more than 50 % after more than 5 years. Major risk factors are high L-Dopa dose, longer disease duration and younger age. Motor response fluctuations are often referred to as ON-OFF oscillations in analogy to a switch – like action whereby individual doses of L-Dopa produce symptom control (= 'ON') which is replaced by the 'OFF' condition when drug effects have worn off and symptoms recur. They reflect the short half-life of L-Dopa, but variations in gastrointestinal absorption and blood-brain barrier transport as well as striatal pharmacodynamics changes also play a role thus producing different patterns of variation in motor response.

L-dopa induced dyskinesias (LID's) are adventitious Involuntary movements that may be choreic, dystonic or mixed in appearance and occur in different temporal association to the L-Dopa response cycle: ON-period dyskinesias are most often choreic in nature and affect the limb, trunk, face and neck, while movements associated with OFF-periods are dystonic and painful cramps chiefly affecting the foot and lower limb. Some patients experience dyskinesias (choreic or mixed) at times of transitioning into an ON-phase or OFF-phase (" biphasic dyskinesias**.** Mechanisms underlying LID's include pre- and postsynaptic mechanisms: loss of dopaminergic nigrostriatal terminals leads to reduced presynaptic dopamine storage capacity and dysregulated DA release and discontinuous delivery of L-Dopa resulting in pulsatile activation of postsynaptic DA receptors. Striatal output activity becomes altered via supersensitivity of DA receptors and structural and molecular changes leading to altered signal processing in striatal neurons. Serotonergic maladaptive plasticity with sprouting of striatal serotonine terminals with ectopic dopamine release as well as excessive glutamatergic activity in corticostriatal and subthalamopallidal projections contribute to altered activity patterns in basal ganglia thalamocortical networks.

TABLE 1: Classification of hereditary parkinsonism.[144,251]

Locus Symbol	Gene locus	*Gene*	OMIM *	Clinical Clues	New designation **
Classical parkinsonism (autosomal dominant inheritence)					
PARK1 or PARK4	4q22.1	*SNCA*	168601; 163890 (*PARK1*) 605543; 163890 (*PARK4*)	Missense mutations (*PARK1*) cause classical parkinsonism. Duplication or triplication of this gene (*PARK4*) cause early onset parkinsonism with prominent dementia	PARK-*SNCA*
PARK8 [4]	12q12	*LRRK2*	607060; 609007	Variations in *LRRK2* gene include risk-conferring variants and disease-causing mutations	PARK-*LRRK2*
PARK17	16q11.2	*VPS35*	614203; 601501		PARK-*VPS35*
"early onset parkinsonism" (autosomal recessive inheritance)					
PARK2	6q26	*PARK2 encoding Parkin*	600116; 602544	Often presents with dystonia, often in a leg	PARK-*Parkin*
PARK6	1p36.12	*PINK1*	605909; 608309	Psychiatric features common	PARK-*PINK1*
PARK7	1p36.23	*PARK7 encoding DJ-1*	606324; 602533		PARK-*DJ1*
PARK19B	1p31.3	*DNAJC6*	615528; 608375	onset of parkinsonism between the third and fifth decades	PARK-*DNAJC6*
"Complex genetic forms" that have parkinsonism as a key clinical feature but also present with atypical, multisystem features or other movement disorders (autosomal recessive inheritance)					
PARK9	1p36.13	*ATP13A2*	606693; 610513	Early onset parkinsonism with complex phenotype (e.g. dystonia, supranuclear gaze palsy, pyramidal signs, cognitive dysfunction) (Kufor-Rakeb syndrome)	PARK-*ATP13A2*
PARK14	22q13.1	*PLA2G6*	256600; 603604	Complex clinical phenotype (NBIA2, PLA2G6-associated neurodegeneration, PLAN) and the	NBIA/DYT/PARK-*PLA2G6*

				majority of cases do not include parkinsonism	
PARK15	22q12.3	*FBXO7*	260300; 605648	Early onset parkinsonism with pyramidal signs and a variable complex phenotype (e.g. supranuclear vertical gaze palsy, early postural instability, chorea, dystonia)	PARK-*FBXO7*
PARK19A	1p31.3	*DNAJC6*	615528; 608375	Juvenile onset parkinsonism; occasional mental retardation and seizures.	PARK-*DNAJC6*
PARK20	21q22.11	*SYNJ1*	615530; 604297	May have seizures, cognitive decline, abnormal eye movements, and dystonia	PARK-*SYNJ1*
PARK23	15q22.2	*VPS13C*	616840; 608879	young-adult onset parkinsonism associated with progressive cognitive impairment leading to dementia and dysautonomia	not yet assigned

* **Phenotype MIM number; Gene/Locus MIM number**

** based on the recommendations of the MDS task force on the nomenclature of genetic movement disorders, which will be regularly updated: MDSGene; available at http://www.mdsgene.org [144,251]

The locus symbols are in accordance with the Online Mendelian Inheritance in Man ® (OMIM ®) catalogue (https://omim.org/). Seven loci, which have been assigned a PARK designation, have a yet unconfirmed relationship to disease (i.e. PARK3, unknown gene on 2p13; Park5, *UCHL1* on 4p13; Park11, *GIGYF25* on 2q37.1; Park 13, *HTRA2* on 2p13.1; Park18, *EIF4G1* on 3q27.1; Park21, *DNAJC13* on 3q22; Park22, *CHCHD2* on 7p11.2) and 3 are classified as risk loci (PARK10 on 1p32; Park12 on Xq21-q25; Park16 on 1q32). Mutations in *TMEM230* on 20p12 have also very recently been described to cause monogenic PD, but causal relationship to disease is still uncertain[145,252].

Abbreviations: MDS = International Parkinson and Movement Disorder Society; NBIA2 = neurodegeneration with brain iron accumulation 2A

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19 **TABLE 2: Imaging biomarkers for used to study Parkinson's disease**

target (technique and/or marker)	findings ins PD	diagnostic potential	research setting
presynaptic nigrostriatal neuron integrity (different PET and SPECT tracers)	• decreased (affecting caudal more than rostral striatum) due to nigrostriatal dopaminergic denervation	• early and differential diagnosis (PD vs. non-degenerative parkinsonism or non-PD tremor)	• sensitive to disease progression • marker for prodromal disease (e.g. RBD and asymptomatic LRRK2 carriers)
striatal dopamine D2/D3 receptors (different PET and SPECT tracers)	• binding increased in putamen in untreated patients with normalisation or slight decrease on dopaminergic treatment	• reduced binding in atypical parkinsonism, but suboptimal diagnostic accuracy	• receptor occupancy studies with dopaminergics
myocardial postganglionic sympathetic innervation (different PET and SPECT tracers)	• decreased cardiac uptake (however normal cardiac sympathetic innervation in early stages of PD in up to 60%)	• differential diagnosis (PD vs. atypical parkinsonism or non-degenerative parkinsonism; results may be confounded by cardiac co-morbidity and several drugs)	• marker for prodromal PD (e.g. RBD)
glucose metabolism (FDG-PET)	• (relative) hypermetabolism of putamen/pallidum and, possibly, thalamus and cerebellum	• early and differential diagnosis (PD vs. essential tremor or atypical parkinsonism)	• global functional level spatial covariance analysis reveals a PD-related pattern of metabolic alterations (PDRP) [e] • progression marker for PD (PDRP) • marker for prodromal PD (e.g. RBD) • surrogate marker for treatment effects (change of PDRP scores)
nigral echogenicity (TCS)	• hyperechogenicity in the area of the SN (can be found in up to 23% of healthy controls)	• early and differential diagnosis (PD vs. atypical parkinsonism or non-degenerative parkinsonism)	• risk marker for PD • marker for prodromal PD (e.g. RBD and asymptomatic LRRK2 carriers)
brain structure (routine sequences of MRI)	• no disease-specific changes in PD, especially in the early disease stages • mild cortical atrophy involving hippocampal and frontal structures as disease progresses	• diagnosis of symptomatic parkinsonism due to underlying CNS pathologies • support differential diagnosis vs. atypical parkinsonism	• characterisation of subtypes and progression of PD (e.g. GM volume analysis, cortical thickness measurements, cortical gyrification)
dorsolateral nigral hyperintensity (DNH) (iron-sensitive sequences:T2* and SWI)	• loss of DNH (suggested to reflect degeneration of nigrosome 1; called also "swallow tail"-sign)		• diagnostic marker for PD • marker for prodromal PD (e.g. RBD)
iron accumulation (iron-	• increased nigral iron content	• differential diagnosis (increased putaminal iron	• potential diagnostic marker for PD

sensitive sequences: T2*, SWI, QSM)		content can help to discriminate atypical parkinsonism vs. PD)	
nigral neuromelanin content (neuromelanin-sensitive MR sequences)	• reduced size, volume and signal intensity of the SN		• diagnostic and progression marker for PD
diffusion metrics [a] (DWI/DTI)	• abnormal nigral diffusion metrics	• differential diagnosis vs. atypical parkinsonism (abnormal diffusivity in putamen and/or infratentorial structures)	• diagnostic and progression marker for PD
complementary brain tissue changes (multimodal MRI) [b]	• abnormal nigral diffusion metrics and abnormal nigral iron content		• diagnostic marker for PD
connectivity (structural with tractography [c] and functional with rs-fMRI [d])	• reduced structural connectivity of the SN with the basal ganglia • decreased coupling in different nigral and striatal brain networks		• diagnostic marker for PD • characterisation of subtypes of PD

[a] includes mean diffusivity (i.e. displacement of molecules and presence of obstacles to diffusion) and fractional anisotropy (orientation of diffusion in structures such as axons in fibre bundles which relates to membranes, myelin, longitudinal filaments, and cytoskeleton) and free-water measurements (i.e. the fractional volume of unconstrained diffusion); [b] multiple MR parameters sensitive to complementary tissue characteristics; [c] obtained by DTI, and measures are diffusion metrics within the tracts, number of tracks and connection probability between regions; [d] assessed with fMRI to ascertain temporal correlations of low-frequency, spontaneous BOLD signal fluctuations between spatially remote regions; [e] characterized by putamino-/pallidothalamic and pontine hypermetabolism, along with hypometabolism in the pre-motor, supplementary motor, and parietal association cortex;

Abbreviations: CNS = central nervous system; DNH = dorsolateral nigral hyperintensity; DWI = diffusion weighted imaging; DTI = diffusion tensor imaging; FDG = ^{18}F-fludeoxyglucose; GM = gray matter; PD = Parkinson disease; PET = positron emission tomography; PDRP = PD-Related Metabolic Spatial Covariance Pattern; QSM = quantitative susceptibility mapping; RBD=REM sleep behaviour disorder; rs-fMRI=resting state fMRI; SN = substantia nigra; SPECT =single-photon emission computed tomography; SWI = Susceptibility weighted imaging; TCS = transcranial brain parenchyma sonography

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35 **TABLE 3: Overview of potential biochemical biomarkers in PD[66,146,150]**
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Biomarker	Results in PD	Comments *
Blood (or Serum/Plasma)		
BDNF	↓	• decreased levels in early disease stages with an increase of levels with progression of the disease more severe motor and cognitive dysfunction with higher levels compared to patients with lower levels
IGF-1	↑	more severe motor and cognitive dysfunction with higher levels
Uric acid	↓	• reduced risk of PD development with higher levels less severe motor and non-motor dysfunction with higher levels
a-syn	↑	divergent results probably due to the different methods to detect α-synuclein in plasma
EGF	↓	increased risk for cognitive impairment in PD with lower levels
APOA1	↓	• increased risk for PD with lower levels • earlier age at PD onset with lower levels greater motor severity with lower levels
Cytokines	altered	• increased TNF-a, IL-6, IL-1, IL-2, IL-10 potentially prognostic biomarkers
CSF		
t-a-syn	↓	• considerable overlap between PD and controls when measuring total a-syn levels • lower levels in PD patients with non-tremor-dominant phenotype [1] • lower levels in patients with cognitive dysfunction
p-a-syn	↑	• less overlap between PD and controls compared to total a-syn levels
o-a-syn	↑	• less overlap between PD and controls compared to total a-syn levels
DJ-1	↓	• seems to be stable over disease stages
GCase activity	↓	• if combined with a-synuclein testing diagnostic accuracy of a-synuclein for PD vs. controls can be increased
Aß-42		• more severe non-motor dysfunction with lower levels

t-tau	↓	• more severe non-motor dysfunction with increased t-tau/Aβ1-42 and t-tau/α-syn ratios
p-tau	↓	•
NFL	=	• increased levels in MSA and PSP compared to PD [2]
faeces		
Microbiota	altered	• significant differences of gut microbiota composition in PD vs. controls • inconsistent differentially abundant taxa between studies • decreased fecal SCFA concentrations (i.e. one main metabolic product of gut bacteria) in PD vs. controls • inconsistent associations of microbiota with clinical variables

[1] sensitivity of 88% and specificity of 40% in separating PD from controls according a recent meta-analysis

2 with a standardized mean difference of 1.60 and 2.04 in MSA and PSP vs. PD according to a recent meta-analysis

*Compared to healthy controls unless specified

Abbreviations:

BDNF=Brain-derived neurotrophic factor; IGF-1=insulin-like growth factor; a-syn=alpha synuclein; EGF=epidermal growth factor; ApoaA1=apolipoprotein A1; t-a-syn=total alpha synuclein; p-a-syn=phosphorylated alpha synuclein; o-a-syn= alpha synuclein oligomers; Aβ1-42=amyloid-beta1-42;t-tau=total tau; p-tau=phosphorylated tau; NFL= neurofilament light chain; IL=interleukin; SCFA = short chain fatty acids; TNF= tumor necrosis factor

TABLE 4: Non-Dopaminergic Pharmacological Treatments in the motor symptoms of Parkinson's Disease[182]			
Indication	**Target / Mechanism of Action**	**Examples**	**Comments**
Motor fluctuations and parkinsonism	Adenosine A2A receptor antagonists	Istradefylline, Preladenant, Tozadenant	Istradefylline approved in Japan and under further evaluation elsewhere; Preladenant – development ceased; Tozadenant – Phase IIb
	Non-selective adenosine antagonist	Caffeine	
	Mixed including inhibition of sodium/ calcium channels and monoamine oxidase-B (MAO–B) activity	Safinamide , Zonisamide	Safinamide - approved in Europe, under review at FDA; Zonisamide - approved for use in Japan
Tremor	Anticholinergics	Many available	Often poorly tolerated; Newer more selective muscarinic antagonists under development
	Mixed antagonist: 5-HT2A, 5-HT2B, 5-HT2C, 5-HT1A, M1, M4, H1, α1, α2, D2, D4	Clozapine	Not approved for this indication; requires hematological monitoring
Levodopa-induced dyskinesia	NMDA antagonists	Amantadine, Dextromethorphan	Amantdine in routine clinical use, Extended-release formulation of amantadine successfully completed phase 3; Dextromethorphan/Quinidine combination (AVP-923) – under study (quinidine is a CYP2D6 inhibitor)
	Mixed antagonist: 5-HT2A, 5-HT2B, 5-HT2C, 5-HT1A, M1, M4, H1, α1, α2, D2, D4	Clozapine	Single pos DB trial; Not approved for this indication; requires hematological monitoring
	mGluR5 (metabotropic glutamate 5) receptors	Mavoglurant (AFQ056), Dipraglurant (ADX48621)	Develoment of Mavoglurant stopped after failed phase IIb trial, Dipraglurant in phase II
	α1 adrenergic receptor and 5-HT1A agonist	Buspirone	Clinically available as antidepressant, Phase III in PD with LID ongoing

	Leviteracetam	Binds synaptic vesicle protein 2A; reduces neurotransmitter release	Mixed results in DB trials
	Combined 5-HT1A and 5-HT1B agonist	Eltoprazine	Phase II ongoing
	Selective a α7- nAChR partial agonist	AQW051	Phase II completed, results unavailable
Gait disorders, falls and freezing of gait (FOG)	Procholinergic therapy (Cholinesterase inhibitors)	Donepezil, Rivastigmine; Other cholinergic agents (e.g., Varenicline, a nicotinic agonist) under study	Variable, mild effects
	Noradrenaline reuptake inhibitor	Methylphenidate	Variable effects on FOG using high doses (e.g., 80 mg/d)

Abbreviations:
IBAT = ileal sodium-dependant bile acid transporter
OL = Open Label; DB = Double-Blind (placebo controlled RTC)