Part I: 2017 UPDATE ON OUR CURRENT UNDERSTANDING OF PARKINSON DISEASE

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In the last decade, advancements in pathology, genetics, biomarkers, and imaging, amongst others, have shaped our current and evolving understanding of Parkinson's disease (PD). The aim of this program is to provide a relevant and coherent overview of our most current understanding of PD.

1) **Epidemiology** – PD is the second most common neurodegenerative disease after Alzheimer's disease, affecting about 10 million people worldwide. PD prevalence increases steadily with age. Based on a meta-analysis, PD prevalence rises from 107/100,000 in people 50-59 years of age to 1,087/100,000 in those 70-79 years of age. A systematic review of incidence studies reported a significantly greater incidence of PD in men than in women. One possible explanation for this gender difference is neuroprotection from higher estrogen activity in women.[1] Meta-analyses of different studies found a lower prevalence in Africa and Asia than in North America or Europe. However, geographic variations of prevalence in PD are difficult to analyze and interpret due to differences in population demographics and study methodologies.

2) **Etiology** – The etiology of PD is not entirely understood. In the last decade, increasing evidence supports the notion that PD is a multifaceted disease resulting from complex interactions between genetic and environmental factors giving rise to progressive neurodegeneration in susceptible parts of the brain.

3) **Genetics** – A genetic predisposition has been recognized in PD in the last 20 years. Since the discovery of the first disease-causing mutation in the *SNCA* gene in a large Italian family with autosomal-dominant PD in 1997, investigations into the role of genetics in PD have grown exponentially. The spectrum of genetic basis of PD is broad, encompassing highly penetrant Mendelian inheritance patterns and multifactorial inheritance resulting from genetic susceptibility variants modulated by epigenetic and environmental factors. Presently known Mendelian forms of PD with autosomal dominant and recessive inheritance account for less than 10% of PD cases.

a) *Autosomal dominant and autosomal recessive:* SNCA and LRRK2 are the two dominantly inherited genes that have been studied most in depth and received the most attention, while other genes and loci are more recently discovered with less well-formed data that require more genetic validation. A base pair change in SNCA gene was the first mutation identified as causing PD in 1997. Subsequently multiplication mutations in the SNCA gene were described, with the number of copies of the SNCA gene appearing to correlate with disease severity. The significance of the SNCA gene is related to the subsequent discovery of the encoded protein alpha-synuclein (α-Syn), which is the major component of Lewy bodies, and the pathological hallmark of PD. Animal models utilizing SNCA mutations have led to valuable insights into its pathogenic properties. Detection of α-Syn in blood and cerebrospinal fluid are currently under investigation as an early biomarker for PD. Mutations in LRRK2 are the most frequent cause of dominantly inherited PD. It accounts for 2% of all PD, and 5% of familial cases. Its prevalence can reach up to 30-40% in Ashkenazi Jews and North African Arab communities with PD. The clinical presentation of the parkinsonian phenotype caused by LRRK2 is often indistinguishable from sporadic PD. However, its pathological features are more heterogeneous and atypical, with a lack of Lewy bodies in some cases.[2] Similarly, parkinsonism with little or no Lewy pathology has also been observed in PARK2-related PD. These pathological findings challenge the traditional view that abnormal α-Syn deposition in the substantia nigra pars compacta (SNc) is essential to the neurodegenerative process and development of motor symptoms in PD. This has pushed forward a global effort to reevaluate both the pathologic criteria for and definition of PD. Mutations in Parkin, PINK1, and DJ-1 have been identified to cause autosomal recessive forms of PD. These genetic forms of PD are generally characterized by pure parkinsonism, early onset, slow progression and good response to levodopa. Other genes (PARK9, PARK14, PARK15) with autosomal recessive inheritance have been associated with more complex phenotypes and additional neurological findings such as hyperreflexia, spasticity, dystonia, and dementia.

b) *Glucocerebrosidase mutations:* Clinical observations of frequent occurrence of PD in relatives of patients with Gaucher disease, an autosomal recessive lysosomal storage disorder, led to the discovery that heterozygous mutations in glucocerebrosidase (GBA) gene increase PD risk by 5-fold.[3] Recently, a bidirectional pathogenic relationship has been suggested between α-Syn accumulation and reduced GBA activity.[4]

c) *Mitochondrial mutations:* Mitochondrial dysfunction has been recognized as a part of PD pathogenesis. Parkinsonism can result from intravenous injection of MPTP, a potent inhibitor of the mitochondrial respiratory chain, observed in illicit drug users in 1970s and later confirmed in animal models. Frequent mitochondrial DNA mutations and reduced mitochondrial function in complex I of the respiratory chain have been detected in the SNc of PD brains, further implicating mitochondrial dysfunction in PD.[5]

d) *Genome-wide association studies (GWAS) and Epigenetics:* In the last few years, GWAS has emerged in an attempt to assess genetic variability across the genome and their contribution to disease risk. These studies have identified more than 20 susceptibility loci associated with increased risk of PD.[6] There has also been a growing body of research in epigenetics examining the role of epigenetic modifications such as DNA methylation, histone acetylation, and micro-RNAs in PD pathogenesis. These modifiers are thought to act as potential mediators between environmental exposure and genes, allowing changes in phenotype without change in genotype. Expression of SNCA is regulated by DNA methylation. Hypomethylation of SNCA has been shown in the brains of PD patients. The observation of lower PD occurrence in women compared to men may be explained by methylation patterns of sex-specific genes such as MAPT. Various levels of miRNA expression have been associated with sporadic PD.

4) **Environmental factors –** Many exposures have been examined in relation to PD. Studies have reported an inverse association between PD and smoking and also coffee drinking. History of head trauma with loss of consciousness has also been associated with PD. Pesticide exposure has been associated with higher PD risk. An inverse association was found between serum uric acid level or gout and PD.[7] An inverse relationship between PD and total vitamin D levels has also been suggested.[8] Higher total cholesterol levels may be associated with lower PD risk, but the relationship between statin use and PD has been inconsistent. The causality of these associations remains controversial due to possible reverse causation and information bias.

5) **Pathophysiology** – PD is characterized by intraneuronal protein accumulation in the form of Lewy bodies and Lewy neurites in the SNc and other regions of the central and peripheral nervous system (PNS). Alpha-synuclein is the principal component of Lewy pathology and thought to play an important role in PD pathogenesis. The significance of α-Syn in PD originates from discovery of rare Mendelian forms of PD caused by mutations in SNCA. Genetic variations in SNCA are linked to an increased risk for sporadic PD. With advances in immunostaining, the extent of Lewy pathology in PD is now known to be more widespread than originally thought. Neurodegeneration with Lewy pathology is also found in the nucleus basalis of Meynert, locus coeruleus (LC), median raphe, and nerve cells in the olfactory system, upper and lower brain stem, cerebral cortex, spinal cord, and peripheral autonomic system. Perhaps one of the greatest pathological advancements in PD was when Braak and colleagues examined α-Syn distribution in brains of PD patients and controls. They proposed a sequential pattern of Lewy pathology distribution, beginning in the olfactory system and dorsal motor nucleus of the vagus, progressing to involve the peripheral autonomic nervous system, extending to involve SNc in the mid-stage of the disease, later involving the upper brainstem, and finally affecting the cerebral hemispheres. These findings offered valuable insights and shaped our understanding of where and when PD may originate and how it evolves. The findings of Lewy bodies in the olfactory cells and autonomic nerves of the heart and GI tract prior to neurodegeneration in SNc and the development of classic motor symptoms of PD support the concept of a *prodromal (premotor) phase* of PD.

The Prion Hypothesis – The pathological spreading scheme in the brains of PD raised the possibility of prion-like mechanism of α-Syn in disease progression. Autopsy studies in PD patients with fetal brain tissue grafts found Lewy pathology similar to that of PD in the grafted neurons more than 10 years after the transplant procedure, suggesting a possible transmissible nature of α-Syn. Under certain circumstances, α-Syn undergoes conformational change from α-helical structure to β-sheet-rich fibrils, similar to prion proteins. Recent studies using animal models have demonstrated a “seeding” phenomenon of α-Syn fibrils inducing endogenous α-Syn protein to misfold, aggregate, form Lewy body-like inclusions, and cause neuronal death.[9] The prion hypothesis has challenged the traditional way that we view PD.

The Gut Microbiota (GM) – Human GM have now been accepted as a potential modulator of cognition, learning, and behavior, and can directly or indirectly modify brain neurochemistry. GM can influence dopamine turnover,

dopaminergic cell expression, striatal gene expression, etc. The GM's composition is altered in PD, and that this dysbiosis has been related to motor fluctuations.[10] More studies are needed to establish a cause and effect relationship between GM and PD.

Finally, our recent advances on PD genetics and pathophysiology have researchers looking at new targets to delay disease progression, such as immunotherapies for synucleinopathies (i.e. alpha synuclein antibodies),[11] and the modulation of glucacon-like peptide-1[12] and glucocerebrosidase/glycoceramide activity.[13]

6) **Diagnosis and Biomarkers** – It has been increasingly recognized that PD has a long prodromal phase during which early symptoms can occur years before the appearance of motor parkinsonism. These early symptoms are often non-motor features that parallel with the early neuropathological stages proposed by Braak and colleagues. These non-motor features include olfactory dysfunction, REM sleep behavioral disorder, constipation, anxiety, and depression.

Our current method of diagnosing PD during life remains clinical, while definitive diagnosis is obtained through pathologic confirmation of α-Syn deposition and neurodegeneration in the SNc. Due to overlapping symptoms with other neurodegenerative disorders and less well defined symptoms and signs in early disease, misdiagnosis is not uncommon. Clinical diagnostic accuracy ranges 75-95% depending on disease duration and stage, and clinician expertise. Using UK Parkinson's Disease Society Brain Bank Research Center criteria for PD, the pooled diagnostic accuracy was 82%.

New Diagnostic Criteria – The International Parkinson and Movement Disorder Society (MDS) recently created a task force to update the disease definition and proposed MDS Clinical Diagnostic Criteria for PD in 2015 (Table 1). The MDS-PD criteria incorporated non-motor symptoms while retaining the central features of parkinsonism as bradykinesia in combination with either rest tremor, rigidity, or both. After documentation of motor parkinsonism, MDS-PD criteria proposed a list of absolute exclusions and red flags that argues against the diagnosis of PD, and supportive criteria that argue in favor of PD as the etiology of parkinsonism. Two ancillary diagnostic tests, olfactory loss and metaiodobenzylguanidine (MIBG) scintigraphy, were deemed reliable, with specificity >80%; these can be used as a supportive criteria. Two levels of diagnostic certainty based on these positive and negative factors were proposed: *clinically established PD* and *clinically probable PD*.[14]

Table 1: MDS Diagnostic Criteria for PD

Absolute exclusion criteria	
Unequivocal cerebellar abnormalities	
Downward vertical supranuclear gaze palsy, or selective slowing of downward vertical saccades	
Diagnosis of probable behavioral variant FTD or primary progressive aphasia within first 5 years of disease	
Parkinsonian features restricted to the lower limbs for > 3 years	
Treatment with a dopamine receptor blocker or a dopamine-depleting agent consistent with drug-induced parkinsonism	
Absence of observable response to high-dose levodopa despite at least moderate severity of disease	
Unequivocal cortical sensory loss, clear limb ideomotor apraxia, or progressive aphasia	
Normal functional neuroimaging of the presynaptic dopaminergic system	
Documentation of an alternative condition known to produce parkinsonism and plausibly connected to the patient's symptoms	
Supportive criteria	**Red flags**
Clear and dramatic beneficial response to dopaminergic therapy	Rapid progression of gait impairment requiring regular use of wheelchair within 5 years of onset
Presence of levodopa-induced dyskinesias	A complete absence of progression of motor symptoms or signs over ≥5 years, unless stability is related to treatment
Rest tremor of a limb, documented on clinical exam	Disproportionate anterocollis or contractures of hands/feet within the first 10 years
Presence of either olfactory loss or cardiac sympathetic denervation on MIGB scintigraphy	Severe autonomic failure in first 5 years of disease: orthostatic hypotension, severe urinary retention or incontinence
	Inspiratory respiratory dysfunction
	Recurrent falls because of impaired balance within 3 years of onset
	Early bulbar dysfunction within the first 5 years
	Absence of any of the common nonmotor features despite 5-year disease duration
	Otherwise unexplained pyramidal tract signs
	Bilateral symmetric parkinsonism

Biomarkers – There is no definitive validated biomarker for PD at this time. A number of candidates are undergoing evaluation, however, including fluid and tissue analysis, genetic susceptibility, clinical evaluations such as olfactory testing, and neuroimaging. Ongoing efforts to identify genetic risk factors and their association with disease pathophysiology can help to elucidate the underlying cause of disease and identify at-risk populations. The SNCA gene and its protein α-Syn have been among the most investigated biomarker candidates. The evidence that PD involves the peripheral nervous system by Braak et al. sparked a search for biomarkers from peripheral sites. Based on the finding that cardiac sympathetic nerve fibers are affected in early PD, MIBG myocardial scintigraphy has been found to be a useful tool in differentiating PD from other parkinsonism with high sensitivity and specificity. Alpha-synuclein has been detected in CSF, blood, saliva, urine, skin, GI tract and submandibular gland tissue fro PD patients.[15] Development of a tissue biopsy test has been challenging, however, due to technical issues surrounding tissue sampling, collection, and suboptimal specificity.

Several neuroimaging modalities have been developed to provide important information on brain structure and function in PD. Positron emission tomography (PET) and single photon emission tomography (SPECT) utilizing radiolabeled tracers have allowed functional assessment of the nigrostriatal pathway. Neurodegeneration in the substantia nigra leads to decreased striatal density of presynaptic dopaminergic nerve terminals and dopamine transporters (DAT), which can be reflected by reduced ligand binding on DAT SPECT imaging. SPECT with DAT radiotracers can help to distinguish neurodegenerative parkinsonism from non-neurodegenerative parkinsonism. However, this test is unable to differentiate different etiologies of neurodegenerative parkinsonism. PET imaging measuring cerebral glucose metabolism and cerebral blood flow offers insights into regional brain activity, and may have some potential application for the differential diagnosis of parkinsonian syndromes.[16] Transcranial sonography (TCS), a noninvasive and low-cost ultrasound imaging method, has shown potential usefulness in the clinical diagnosis of PD by assessing the echogenicity of the SNc. Hyperechogenicity of the SNc has been found in up to 96% of PD patients, and has been linked to increased PD risk. However, its usefulness is limited by operator's skill and requirement for an adequate acoustic window.[17] Conventional MRI in PD is mainly used to exclude other possible etiologies. Novel MRI techniques have been developed to evaluate the SNc in PD. Based on the finding of loss of neuromelanin-containing neurons in the SNc and locus coeruleus (LC) in early PD, neuromelanin-sensitive MR imaging was developed for better visualization of the SNc and LC. Reduction in SNc and LC volume has been observed in PD patients compared with control subjects using this technique.[18] Autopsy studies have shown increased iron deposition in the SN in PD; this finding has also been observed using MR studies.[19]

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