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Invited review

Advances in the genetics of Parkinson's disease[1]

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Key words

Abstract

Parkinson's disease (PD) is a neurodegenerative disorder affecting a significant proportion of the ageing population. The etiology is unknown and it is likely due to a multifactorial interaction of genes and the environment on the background of ageing. Findings in the last decade suggest that the contribution of genetics to familial forms of PD is much greater than previously appreciated. Twelve loci are now associated with highly penetrant autosomal dominant or recessive PD, and causative mutations have been identified in eight genes with mutation carriers often characterized by a phenotype indistinguishable from idiopathic disease. To date, PD pharmacotherapy is symptomatic only and does not slow disease progression. Understanding how genetic mutations cause familial PD is likely to clarify molecular mechanisms underlying PD in general and will provide a guide for the development of novel therapies, both preventative and palliative, applicable to all forms of parkinsonism. This review outlines the advances in the study of the genetic background of PD and their possible clinical implications.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder with an overall prevalence of approximately 1.8% of the population over the age of 65 years[1]. PD is characterized by a clinical phenotype consisting of resting tremor, bradykinesia, rigidity, and postural instability, typically asymmetric at onset, gradually progressive, and responsive to dopaminergic therapy[2]. Pathologically, PD is characterized by the loss of dopaminergic neurons in the substantia nigra with intracytoplasmic inclusions containing aggregated α-synuclein as well as other substances (Lewy bodies) and Lewy neurites in many other brain regions as well as in the remaining intact nigral neurons[3]. Motor symptoms are believed to result from the progressive deficiency or dysfunction of dopaminergic neurons in the substantia nigra, regardless of etiology[4].

Relatively little is known regarding the mechanism of PD pathogenesis, in particular the apparent susceptibility of nigral neurons to degeneration. It has been suggested that the selective loss of dopaminergic neurons and the accumulation of α-synuclein is influenced by defects in the ubiquitin-proteasomal system (UPS), mitochondrial dysfunction, and the impairment of mechanisms protecting from oxidative stress and apoptosis[5–7]. These defects in intracellular mechanisms appear to result from a combination of environmental risk factors and genetic susceptibility superimposed on slow, sustained neuronal dysfunction due to advancing ageing.

The contribution of hereditary factors to the etiopathogenesis of PD was proposed well over a century ago, when Leroux[8] and Gowers[9] each noted that a significant percentage of PD patients had an affected family member. A family history of PD is indeed second only to age as a risk factor for the disease[10,11], although familial aggregation of the disease does not necessarily indicate a genetic component, as disease risk may be increased from shared environmental factors. Cross-sectional twin studies reporting similar concordance rates in monozygotic and dizygotic twins challenged the significance of a genetic contribution to PD[12,13], yet suggested that genetic factors may be important when disease begins before the age of 50 years[12]. However, longitudinal twin studies using ^{18}F-dopa positron emission tomography (PET), highlighting clinically presymptomatic

dopaminergic loss, reported a concordance rate of 75% in monozygotic twins, compared to 22% in dizygotic pairs, regardless of age at onset[14] supporting a genetic etiology and suggesting that the initial low concordance reported in MZ twins was likely the result of age-related disease penetrance[15].

The past decade has seen a major breakthrough in the genetics of PD, with the identification of 12 genomic regions and pathogenic mutations in 8 genes unequivocally linked to familial PD. The monogenic forms of PD display autosomal dominant and autosomal recessive modes of inheritance, and account for 1%–3% of late-onset disease and approximately 20% of young-onset disease[16,17].

Gene products linked to monogenic forms of PD

Dominantly-inherited PD Dominant forms of familial PD are caused by mutations in *SNCA*[18,19], *UCH-L1*[20], and *LRRK2*[21,22] genes, with probable gain of function effects. Mutations in these genes have also been identified in sporadic late onset PD, suggesting that their protein products might have implications for mechanisms underlying the common idiopathic forms of PD (Table 1).

SNCA (PARK1/4) Mutations in the *SNCA* gene, encoding α-synuclein, were the first familial PD-associated mutations reported, identified in a large kindred with autosomal dominant PD[18]. Three point mutations that segregate with familial PD have now been identified, Ala53Thr[18], Ala30Pro[23], and Glu46Lys[24].

SNCA gene mutations are rare, accounting for less than 1% of Parkinson disease in the general population[25]. PD patients carrying *SNCA* mutations have clinically typical PD, with levodopa responsiveness, although disease onset is earlier than in patients with idiopathic PD, and progression appears to be more rapid. Neuropathological findings are similar to those in idiopathic disease, with cell degeneration, Lewy bodies, and neurites. Increased dosage of the wild-type *SNCA* gene, by either duplication or triplication, has been associated with PD in unrelated families[19,26]. *SNCA* dosage appears to be correlated with phenotypic severity and age of onset of the disease, with triplication causing rapidly progressive parkinsonism with an early age at onset and early death[19], while *SNCA* duplication resembles idiopathic PD, with a late age at onset and slower disease progression[26]. Common *SNCA* gene variants, including a dinucleotide repeat sequence (REP1) within the promoter, have been implicated in increased risk for idiopathic PD[27,28]. Recently, a large meta-analysis confirmed the association between allele-length variability in the dinucleotide repeat and increased PD risk[25].

Table 1. Monogenic forms of PD

Locus	Chromosome location	Gene	Inheritance	Age of onset (y) Phenotype	Lewy Bodies	Putative function	Reference
PARK1/4	4q21-q23	*SNCA/α-synuclein*	AD	<50, B, R	+	Unknown synaptic function/possibly vesicle trafficking	[18,19]
PARK2	6q25-q27	*parkin*	AR	<50,B,R, T, D slow progression	+/–	E3 ubiquitin ligase	[55]
PARK3	2p13	Unknown	AD	>50, B,R, T	+		[151]
PARK5	4p14	*UCH-L1*	AD	49,51, B,R, T	Not reported	Ubiquitin C-terminal hydrolase	[20]
PARK6	1p35-p36	*PINK1*	AR	<50,B,R, T, slow progression	Not reported	Mitochondrial serine/threonine kinase	[65]
PARK7	1p36	*DJ1*	AR	<50, B,R,T slow progression	Not reported	Chaperone, oxidative stress response?	[57,70]
PARK8	12p11.2-q13.1	*LRRK2*	AD	>50, B,R, T	+/–	Unknown protein kinase	[35]
PARK9	1p36	*ATP13A2*	AR	Juvenile onset	Not reported	Lysosomal type 5 ATPase	[58,75]
PARK10	1p32	Unknown	Unclear/AD?	Typical PD	Not reported		[152]
PARK11	2q36-37	Unknown	Unclear/AD?	Typical PD	Not reported		[153]
PARK12	Xq21-q25						[154]
PARK13	2p12	*OMI/HTRA2*	AD	>50, B, R, T	Not reported	Serine protease	[79]

AD , autosomal dominant; AR, autosomal recessive; B, bradykinesia; R, rigidity; T, tremor; D, dystonia.

The normal function of α-synuclein is not fully understood, but evidence suggests it plays a role in vesicular function and may also have chaperone properties[29]. α-Synuclein is a major component of Lewy bodies in both familial and sporadic PD[30]. The three *SNCA* point mutations alter the properties of the α-synuclein protein, leading to increased protein aggregation, likely critical to Lewy body formation and PD pathogenesis[31].

Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1; PARK5) The ubiquitin–proteasome system has been repeatedly implicated in PD, and the analysis of genes encoding proteins in this pathway in 72 PD families revealed a single missense mutation (Ile93Met) in the *ubiquitin carboxy-terminal hydrolase L1 (UCH-L1)* gene[20].This mutation was detected in a single sibling pair, the only PD patient carriers of this mutation identified to date. PD in these two patients resembled idiopathic disease, and neuropathology was not available. Subsequent screenings in multiple PD cohorts suggest that a common polymorphism, Ser18Tyr, in the *UCH-L1* gene has a protective effect in PD[32]. *UCH-L1* is abundant in the human brain and is a component of Lewy bodies[33]. This, together with its involvement in the ubiquitin-proteosomal system[34], supports *UCH-L1* as a convincing candidate gene for PD.

LRRK2 (PARK8) *PARK8* was originally detected in the large Japanese Sagamihara kindred with autosomal dominant parkinsonism[35], and mutations in *leucine-rich repeat kinase 2 (LRRK2)* were subsequently identified in a number of families with late-onset autosomal dominant parkinsonism, including the Sagamihara kindred[21,22,36]. Interestingly, mutations in this gene have also been found in late-onset PD patients without a known family history of PD[37,38], suggesting that even late-onset apparently sporadic PD might have a significant genetic component[39].

Mutations in the *LRRK2* gene are the most common genetic determinant of PD identified to date[16]. Thus far, at least 20 *LRRK2* mutations have been implicated in *LRRK2*-linked PD estimated to account for approximately 7% of familial PD cases and up to 3% of apparently sporadic disease. While eight mutations which lead to amino acid substitutions seem to be pathogenic, segregating with PD, the others, found in small families or single individuals, are considered putatively pathogenic[40,41]. Gly2019Ser, the most common pathogenic *LRRK2* mutation, has been associated with disease at varying frequencies in populations worldwide, and is particularly prevalent among Ashkenazi Jews[42–44] and North African Arabs[45,46]. Recently, a significant association has been reported between the *LRRK2* Gly2385Arg missense variant and PD in Asian populations. Originally iden-

tified as putative pathogenic mutation in a small Taiwanese PD family[40], *LRRK2* Gly2385Arg was subsequently reported as a common polymorphism and not a pathogenic mutation, significantly more frequent among patients with PD than controls in ethnic Chinese populations from Taiwan and Singapore[47–50]. This variant has also been suggested as a risk factor for sporadic PD in the Japanese population[51]. Considering the size of the ageing Asian population, the *LRRK2* Gly2385Arg variant is probably the most frequent genetic risk factor for PD worldwide[49,50].

LRRK2-associated PD is clinically indistinguishable from idiopathic disease[52], although substantial variations in neuropathological findings has been reported, including pure nigral degeneration without LB and nigral degeneration associated with brainstem LB typical of PD[22,53].

LRRK2 is a large gene encoding Lrrk2, predicted to contain five functional domains which are believed to be involved in multiple functions, including substrate binding, protein phosphorylation, and protein–protein interactions. All of the identified mutations occur in predicted functional domains[41]. The most frequent pathogenic mutation, Gly2019Ser, occurs in the kinase domain and has been shown to increase kinase activity[54].

Recessively-inherited PD Autosomal recessive (AR) forms of familial PD are caused by homozygous and compound heterozygous mutations in *parkin*[55], *PTEN-induced kinase 1 (PINK1)*[56], *DJ1*[57], and *ATP13A2*[58]. These relatively rare mutations, with probable loss-of-function mechanisms, are a significant cause of early-onset PD (< age 45 years; Table 1).

Parkin (PARK2) Mutations in the *parkin* gene were first described in consanguineous Japanese families with autosomal recessive juvenile parkinsonism (AR-JP)[55]. *Parkin* mutations are the most common cause of AR-JP PD and have been estimated to account for close to 50% of familial patients with recessive inheritance and disease onset before the age of 45 years, as well as 18% of the early-onset apparently sporadic cases[59]. Over 100 mutations in *parkin*, including exonic deletions, insertions, and point mutations have been observed in patients of all ethnic backgrounds[60]. It has been suggested that *parkin* alterations may also manifest in an autosomal dominant pattern of disease inheritance, with a single alteration possibly increasing the susceptibility for PD[61–63].

The clinical phenotype of *parkin*-associated PD varies, although it most commonly resembles idiopathic PD. Features characteristic of *parkin* disease include early onset, symmetrical motor symptoms, dystonia, improvement of symptoms after sleep, and hyperreflexia with relatively slow

progression. Neuropathological studies of patients with *parkin* mutations with homozygous exonic deletions show selective cell loss of the nigrostriatal tract and locus ceruleus, with a remarkable absence of Lewy bodies, suggesting that *parkin* disease is a unique entity unlike sporadic PD[16]. α-Synuclein- and ubiquitin-positive inclusions have been reported in patients bearing compound heterozygous deletions and/or mutations[63].

The parkin protein is an ubiquitin E3 ligase preparing target proteins for degradation mediated by the ubiquitin-proteosomal system[64]. Although several putative parkin substrates have been identified, including proteins that are implicated in PD, it is unclear whether there is a single pathological substrate whose accumulation, due to the disrupted enzymatic function of parkin, is responsible for the neuronal death in the substantia nigra[7,60].

PTEN-induced kinase 1 (PINK1; PARK6) The *PARK6* locus at 1p35-36 was mapped in a Sicilian kindred segregating an AR form of PD, with a relatively early age of onset[65], and was confirmed in additional European families[66]. Subsequently, mutations in the *PTEN-induced kinase 1* (*PINK1*) gene were identified in three of the *PARK6*-linked families[56]. Mutations in *PINK1* are a rare cause of early-onset PD, most likely accounting for 1%–2% of cases[39]. *PINK1* mutations have been identified in families from different European and Asian countries as well as in North American families, indicating that mutations in the gene cause PD in a wide range of populations worldwide[31]. Interestingly, *PINK1* mutations have also been found as a rare cause of sporadic early-onset PD[67].

Families with *PINK1* disease had a variability in the age of disease onset, with at least one individual in each of the linked families with an age at onset of younger than 45. In family members with late-onset PD, the disease phenotype was identical to that of idiopathic PD, although it appears that the phenotype varies in different populations, with Asian patients experiencing earlier disease onset and more frequent occurrence of atypical symptoms, such as dystonia at onset, hyperreflexia, and psychiatric disturbances[31]. The neuropathology of *PINK1* has not been described. Although ^{18}F-dopa PET studies have demonstrated nigrostriatal dopaminergic dysfunction in *PINK1* heterozygous carriers[68], as with heterozygous *parkin* mutations, it is not clear if single *PINK1* mutations increase the risk for PD[16].

PINK1 encodes is a highly conserved, widely expressed mitochondrial kinase that has been suggested to protect neurons from stress-induced mitochondrial dysfunction. Mutations in *PINK1* increase cell susceptibility to stress conditions, inducing mitochondrial dysfunction and

apoptosis[56], thus strengthening hypotheses linking PD to impaired mitochondrial activity and oxidative stress[69].

DJ1 (PARK7) *PARK7*, a second locus on 1p, was identified in a consanguineous pedigree from the Netherlands[70], and the *DJ1* gene was mapped to this locus[57]. Two loss of function *DJ1* mutations have been found in PD patients, a deletion of several of exons, preventing DJ1 synthesis, and a point mutation at a highly conserved residue (Leu166Pro) that reduces the stability of DJ1 and promotes degradation through the ubiquitin-proteosomal system, significantly reducing DJ1 levels[71]. *DJ1* mutations are rare, accounting for less than 1% of early-onset PD[71].

DJ1 PD is characterized by early onset, asymmetry, and slow progression with a good response to levodopa. The neuropathology associated with *DJ1* disease has not been described. Heterozygosity does not likely increase susceptibility for PD, as nearly complete loss of DJ1 protein function is necessary to cause the disease[72].

DJ1 encodes a mitochondrial protein that may be an oxidative stress sensor within cells[73], further supporting a link between mitochondrial impairment and the pathogenesis of PD.

ATP13A2 (PARK9) Kufor–Rakeb syndrome is an AR, juvenile-onset multisystemic neurological disorder whose clinical phenotype includes akinetic-rigid parkinsonism with a good response to levodopa, pyramidal tract dysfunction, supranuclear gaze paresis, and dementia[74]. Kufor-Rakeb was originally detected in a consanguineous Jordanian family[74], was mapped to a 9-cM region of chromosome 1p36[75], and later designated *PARK9*. Despite the distinct Kufor-Rakeb phenotype, overlap with the disease linked to other *PARK* loci and the striking response to levodopa in Kufor-Rakeb patients deemed the *PARK9* designation appropriate. The causative gene underlying *PARK9* was recently revealed to be *ATP31A2*[58]. Mutations in the *ATP13A2* gene were identified in a large non-consanguineous Chilean family and in the original Jordanian kindred[58] and were confirmed in a juvenile-onset PD patient from Brazil[76].

ATP13A2 encodes a large protein belonging to the group 5 P-type ATPase class and displays lysosomal localization in overexpression studies[58]. The function and substrate specificity of this protein are currently undefined, although interestingly, *ATP13A2* mRNA is highly expressed in all brain regions, including the substantia nigra, and is upregulated in the human postmortem midbrains from individuals with common idiopathic PD, compared to comparable substantia nigra dopaminergic neurons from controls[58].

Other PARK loci: OMI/HTRA2 (PARK13) Data from animal models have implicated the *Omi/Htra2* gene in

neurodegeneration with parkinsonian manifestations[77,78]. The *OMI/HTRA2* candidate gene was screened in a German PD patient population and in a large ethnically- and gender-matched control sample[79], revealing a novel mutation, Gly399Ser, in 4 PD patients, but in none of the healthy controls. A novel Ala141Ser polymorphism was also associated with PD risk in this population, detected in the heterozygous state in 6.2% of the PD patients compared to 3% of the controls. Heterozygous carriers of both genetic alterations had late-onset PD with typical clinical features and a good response to levodopa therapy.

OMI/HTRA2 encodes the serine protease Omi/HtrA2[80,81]. Interestingly, Omi/HtrA2 has been identified as a component of Lewy bodies in brain samples from pathologically-confirmed PD patients[79]. The Gly399Ser and Ala141Ser mutations result in the defective activation of the proteolytic activity of Omi/HtrA2, compromised mitochondrial function and morphology, and increased vulnerability to cellular stress[79].

Causative genes have not yet been discovered in the dominantly-transmitted *PARK3* locus, the *PARK12* locus on the X chromosome, or for the *PARK10* and *PARK11* susceptibility loci, whose modes of transmission remain unclear.

Pathways of PD pathogenesis: insights emerging from the study of monogenic forms of PD

Although familial forms of PD account for fewer than 10% of all PD cases[82], the delineation of monogenic forms of PD in family studies has allowed insights into the mechanisms of neuronal degeneration, processes likely relevant in sporadic disease as well. The molecular functions of the *SNCA*, *parkin*, *PINK1*, *DJ1*, *LRRK2*, *OMI/HTRA2*, and *ATP13A2* gene products highlight mitochondrial impairment, oxidative stress, and aberrant protein handling as key events in neuronal dysfunction and degeneration.

Compromise in mitochondrial metabolism, particularly due to defects in complex-I, the first complex of the electron transport chain, was suggested in the pathogenesis of PD long before the identification of disease-causing genes[83]. Postmortem studies have shown mitochondrial impairment and oxidative damage in PD brains[84]. Furthermore, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, and paraquat, noted environmental PD risk factors, are complex-I inhibitors that lead to the aggregation of α-synuclein *in vitro* and in animal models[85,86]. The aggregation of α-synuclein, downstream to mitochondrial dysfunction, might overwhelm the UPS, allowing the further, possibly toxic, accumulation of proteins that would otherwise be targeted for

degradation[87]. PINK1, DJ1, and parkin contribute to mitochondrial protection against oxidative stress, and disease-linked mutations in genes encoding these proteins may compromise mitochondrial integrity, resulting in increased levels of free radicals, as well as a failure of cellular energy and subsequently, impaired UPS function. It has been posited that PINK1 and OMI/HTRA2 might share a common pathway in the mitochondrial response to cellular stress and modulation of apoptosis[88]. UPS activity is also influenced more directly by disease-linked mutations in *SNCA*, *parkin*, and *DJ1*. *SNCA* mutations result in misfolded, abnormally-aggregated α-synuclein overwhelming the UPS, while mutant parkin exhibits reduced UPS-mediated substrate degradation. DJ1 has been suggested to function as a molecular chaperone or protease, refolding or promoting the degradation of misfolded or aggregated proteins, with mutant DJ1 decreasing the effectiveness of the UPS[87]. Mutant ATP13A2, retained in the endoplasmic reticulum and degraded by the proteasome, may also result in UPS overload, resulting in toxic α-synuclein aggregation[58]. Emerging evidence also supports the involvement of the lysosomal system in PD pathogenesis. The lysosomal degradation pathway, an alternative mechanism for the degradation of proteins, lipids, and damaged organelles, participates in α-synuclein clearance[89–91]. Thus, lysosomal dysfunction, possibly elicited by mutant ATP13A2 in *PARK9*-linked disease[58] or by the mutant lysosomal enzyme glucocerebrosidase (GBA), which is also implicated in PD risk[92], might result in the accumulation of α-synuclein. Finally, although the cellular functions of the protein kinases LRRK2 and PINK1 are not yet fully understood, they are possibly constituents of a secondary messenger cascade that influences the phosphorylation of proteins that accumulate in end-stage disease[16].

Integration of the multiple, divergent *PARK* loci protein products linked through mitochondrial impairment, oxidative stress, and protein mishandling to the death of dopaminergic neurons, is still schematic, possibly with interplay at multiple levels. Elucidating the function of each gene product and their interactions remains one of the greatest challenges of PD research.

PD candidate genes

Although there has been great progress in the elucidation of monogenic forms of PD, less is known about genetic alterations underlying the common sporadic form of PD. Multiple biologically-plausible candidate genes have been suggested, based on their roles in the proposed pathways of PD pathogenesis, and case-control association studies have been conducted. Candidate genes studied to date,

including mainly genes related to dopamine synthesis, transport, and degradation, detoxification of toxins in dopaminergic neurons, mitochondrial metabolism, and genes encoding essential transcription factors or neurotrophic factors involved in the development of the mesencephalic dopaminergic system, are the basis for several excellent reviews[93–96]. Despite the intense examination of tens of putative candidates, only *monoamine oxidase B* (*MAOB*) >188 bp allele showed significant association with sporadic PD in meta-analysis[93], while 6 additional genes (*DRD2*, *ND3*, *BDNF*, *SNCA*, *UCH-L1*, and *Nurr1*), showing significant association with sporadic PD, were replicated in several studies[94]. Very recently, a global genetics consortium, including published and unpublished data from diverse sites worldwide, revealed that allele-length variability in the dinucleotide repeat sequence (REP1) of the *SNCA* gene promoter is associated with an increased risk for PD[25].

Parkinsonism has been described in patients with Gaucher disease (GD), a recessively-inherited deficiency of the lysosomal enzyme GBA[97]. Clinical observations and neuropathological evidence have implicated mutations in the *GBA* gene in PD susceptibility, although little is understood regarding the cellular and molecular basis of this association [92]. The identification of Lewy bodies in brain samples from *GBA* carriers (both PD and GD patients), together with emerging evidence linking heterozygous *GBA* mutations to diverse synucleopathies[98], suggest that α-synuclein perturbations may be critical to the relationship between *GBA* mutations and PD risk. Recent data have demonstrated the participation of the lysosomal degradation pathway in α-synuclein metabolism[89–91]. Since the lysosome is the site of GBA metabolism, mutant *GBA* might elicit lysosomal disturbance or interfere with receptor binding at the lysosomal membrane, impairing α-synuclein degradation and clearance, predisposing *GBA* mutation carriers to aberrant α-synuclein fibrillization, and possibly α-synuclein-mediated toxicity[92].

Findings from the substantial number of genetic association studies performed to date, claiming or refuting associations between putative PD genes and disease risk, have been compiled and are publicly available on the continuously updated Parkinson's genetics database, PDGene (http://www.pdgene.org/). Similar to the database that comprehensively catalogs all genetic association studies in the field of Alzheimer's disease (www.alzgene.org)[99], PDGene provides a powerful tool for deciphering the genetics of PD. Also of interest is the Parkinson's disease mutation database curated by the Parkinson's Institute, (www.thepi.org/altruesite/files/parkinson/Mutations/new_page_1.html).

The candidate-gene approach to search for PD suscepti-

bility genes has recently been enhanced by the first high-resolution whole-genome association study of PD[100]. This study highlighted 13 polymorphisms, including SNPs, in the *PARK10* and *PARK11* loci, as potentially significant in PD susceptibility. A subsequent large-scale analysis of over 12 000 patients failed to replicate the 13 implicated SNPs[101]. An additional genome-wide association study was performed in 267 PD patients and 270 neurologically normal controls[102], with its raw data publicly available online at the Coriell Institute website (ccr.coriell.org/ninds/). Very recently, the first meta-analysis of genome-wide association datasets in PD was performed, suggesting several candidate SNPs for further studies in PD susceptibility[103]. We anticipate that future large-scale genome-wide association studies will fully extend genome coverage in order to reveal additional common PD susceptibility genes.

Interplay of genetics and environment in PD risk

A complex interaction of multiple genetic and environmental risk factors is likely to be involved in the development of PD, manifesting in clinical symptoms once a threshold of neuronal loss is exceeded. While the last decade has focused on the identification of PD-related genes, PD was conventionally thought of as a disorder with an environmental cause. In the 1980s, the discovery of a MPTP-induced acute parkinsonian syndrome in intravenous drug users[104] encouraged the search for exogenous toxins underlying PD and parkinsonism, particularly compounds toxicologically or structurally similar to MPTP, including pesticides, such as rotenone and paraquat. Rotenone and a combined paraquat–maneb exposure induced PD-like pathology and motor signs in rodents[105,106] and a meta-analysis of 19 studies suggested a significant association between human exposure to pesticides and the development of PD[107]. Paraquat, the pesticide most often implicated as a potential neurotoxicant, is the only pesticide for which a dose-dependent relationship has been reported between lifetime cumulative exposure and increased PD risk[108].

Other proposed risks include agricultural employment, rural living, and consumption of well water, long-term exposure to specific metals, high fat/high calorie diet, occupational exposure to viral (or other) respiratory infections, and inflammation in the brain in early life as a consequence of either brain injury or exposure to infectious agents. To date, data regarding these risk factors are equivocal. Cigarette smoking, coffee/caffeine intake, vitamin E consumption, and non-steroidal anti-inflammatory drugs all appear to lower PD risk[109].

Emerging evidence suggests that the interplay between environmental exposures and genetic factors may modify the risk for disease in susceptible individuals. A number of studies have reported an association between environmental toxins and polymorphic genes coding for enzymes involved in the metabolism of foreign chemicals or the transport or metabolism of dopamine in PD risk. Case control studies reported increased PD risk in carriers of *CYP2D6*[110], *GSTP1*[111], and *SLC6A3/DAT1*[112] allelic variants exposed to pesticides, and more recently, solvent exposure in *GSTM1* null genotype subjects appeared to increase PD risk[113].

Varying prevalence of PD worldwide: genetic or environmental?

The prevalence of PD and PD-associated mutations differ among ethnic groups and geographical locations. Epidemiological studies have suggested that the prevalence of PD is significantly lower in African-Americans[114] and Asians [115] than in Caucasians, with the lowest disease prevalence in mainland China[96]. The disparity in prevalence may be attributed to environmental differences (climate, industrialization, farming practices, and exposure to environmental toxins), cultural differences (calorie/fat intake and consumption of caffeine and tobacco), and genetic differences (polymorphisms and haplotype structure). Regarding Chinese populations, environmental influences appear significant, with a greater PD prevalence in populations in the industrialized Hong Kong and Taiwan than mainland China[96]. Interestingly, genetic changes in the monoamine oxidase B (*MAOB*), dopamine transporter (*SLC6A3/DAT1*), and *SNCA* candidate genes, which are associated with Parkinson's disease in Caucasian populations, have not been demonstrated in Chinese populations[116–118].

The Gly2019Ser mutation in *LRRK2* is another example of a mutation with ethnic variability. The Gly2019Ser change in *LRRK2* exon 41 has been associated with the disease at varying frequencies in Asian, European, and North American populations, and is particularly prevalent among North African Arabs (37% in familial PD patients, 41% in sporadic PD patients, and 1% in controls)[45,46] and in Ashkenazi Jews (29.7%, 26.1% and 26% in familial PD patients, 13.3%, 7.7%, and 10.6% in apparently sporadic PD patients, and 1.3%, 2.0%, and 2.4% in control samples[42–44], respectively). Despite a wide spectrum of ethnic backgrounds, carriers of this mutation share common haplotypes, suggestive of a single-founder effect[38,119,120] or two-founder events[121], with the founder possibly originating in the Middle East[42]. The Gly2019Ser mutation is very rare and does not appear to play a role in the causality of PD in Asian subjects[122–124]. Interestingly,

the *LRRK2* Gly2385Arg alteration was recently shown to be a common risk factor for PD in ethnic Chinese[47–50] and Japanese[51] populations, and appears to be absent in Caucasians[125], highlighting the contribution of specific genetic factors to disease risk in distinct populations.

Pharmacotherapy, pharmacogenetics, and the search for neuroprotective therapy in PD

Dopaminergic neuron degeneration in PD leads to a profound depletion of dopamine, leading to the cardinal motor symptoms that characterize this disease[4]. Current PD therapy is primarily based on neurotransmitter replacement, using levodopa or dopamine agonists[126]. While virtually all patients enjoy a good response to levodopa, one of the criteria for the diagnosis of PD[127], a minority of patients with pathologically-proven PD experience poor or no response. Regrettably, even in levodopa-responsive patients, therapy becomes less effective over time, as the underlying disease progresses. The improvement of motor function gradually diminishes after 2–7 years of therapy, and a significant proportion of patients develop motor response fluctuations in the forms or "wearing off" or "on/off" phenomenon as well as a peak of dose or end of dose involuntary movements called dyskinesias. In the more advanced stages of the disease, levodopa-induced side-effects can be more disabling than the primary symptoms of the disease itself[128]. Young age at onset, disease duration, duration of levodopa treatment, and female sex have been implicated as risk factors for the development of dyskinesias[129]. Drugs from the dopamine agonist group also elicit undesirable side-effects, including extreme somnolence and neuropsychiatric symptoms[126]. Recently, dopamine agonists have been implicated in a severe "dopamine dysregulation syndrome", and in particular, impulse control disturbances like pathological gambling, hypersexuality, bulimia with weight gain, and excessive drive for money spending[130,131]. Association studies have suggested that functional polymorphisms in genes encoding drug-metabolizing enzymes, drug receptors, and proteins involved in pathway signaling might be important factors in the large inter-individual variability regarding the efficacy of dopaminergic therapy as well as treatment-induced motor complications[94,129]. Advances in the study of pharmacogenetics, the effect of variation in human genetics on variation in response to pharmacological treatment, will eventually influence the choice of PD therapy. Ultimately, a future challenge will be the replacement of standard therapies with novel individualized therapeutic regimes, with maximum efficacy and minimum toxicity.

Non-motor symptoms occur commonly in PD and may be

as debilitating as their motor counterparts[126]. These non-motor phenomena, which may occur years before PD diagnosis and frequently complicate advanced disease, include autonomic dysfunction, sleep disturbances, fatigue, mood disorders, and cognitive dysfunction/dementia. These features likely reflect degeneration of non-dopaminergic neurons and may be unaffected or exacerbated by dopaminergic therapies, emphasizing the necessity for additional PD therapeutic options. As the field of PD has possibly reached the limit of symptomatic therapy, the intensive search for neuroprotective therapy based on the protection or rescue of vulnerable neurons and the arrest of disease progression is critical. An appropriate intervention should address the therapeutically challenging non-motor symptoms of PD while prolonging the period of well-controlled motor symptoms. To date, several candidate PD-neuroprotective agents have been tested in clinical trials, although none have unequivocally demonstrated a disease-modifying effect[132,133].

Hypothesis-generating pathogenetic insights from genetic studies also provide the rationale for additional therapeutic approaches, which can be tested in the laboratory setting and perhaps later in mutation-carrier patients and high-risk presymptomatic individuals. Patients with confirmed hereditary PD will likely be a valuable population for the evaluation of neuroprotective candidates and the arrest of motor and non-motor symptom progression. Asymptomatic high-risk individuals, such as carriers of *LRRK2* and other genetic causes of PD, will play a critical role in the longitudinal study of disease progress from preclinical to symptomatic stages. Neuroimaging can be implemented to monitor disease progress and the efficacy of potential neuroprotective candidates in this population, who might also serve as a valuable source for the identification and assessment of additional clinically meaningful biomarkers and risk factors. Ultimately, neuroprotection will aim to capture high-risk individuals in the presymptomatic period, exploiting the valuable therapeutic window of about five years from the onset of neuronal loss to the appearance of clinical signs[134].

If the pathogenic mechanisms in monogenic forms of PD are similar to sporadic disease, therapeutics delineated from the study of mutation carriers might also have relevance for the greater sporadic PD population. We have learned from the intense study of human cancer, which in the last three decades have seen the thorough analysis of hundreds of cancer-related genes, that there is a tremendous gap between molecular discoveries and the establishment and approval of novel effective therapy. On the other hand, molecular studies and insight into cellular and molecular pathways un-derlying the spectrum of human tumorigenesis have generated multiple effective anticancer treatments, including Trastuzumab (Herceptin) in breast cancer[135] and Imatinib (Glivec) in chronic myelogenous leukemia[136]. Hopefully, the identification, establishment, and approval of PD-neuroprotective therapy will be much shorter.

Two putative PD treatment strategies might target the products of the autosomal dominant *SNCA* and *LRRK2* genes. α-synuclein is likely the most promising target for sporadic PD due to its deposition into Lewy bodies. α-synuclein aggregation is believed to be critical for neuronal toxicity, and an effective therapy might lower α-synuclein levels. The controversy regarding which forms of aggregated α-synuclein mediate toxicity, soluble oligomers, or highly insoluble fibrils must first be resolved. Transgenic mice deficient for α-synuclein or expressing mutant (Ala30Pro, Ala53Thr, or both) or wild-type α-synuclein have been generated. Interestingly, while α-synuclein knockouts did not demonstrate any detectable abnormalities other than an alteration of dopamine release in response to rapid stimulation[137], they showed resistance to the dopaminergic toxin MPTP[138], implicating α-synuclein in the pathogenic mechanism that leads to MPTP-induced parkinsonism. While disappointingly, the overexpression models did not display dopaminergic neuronal death in the substantia nigra, they recapitulated a spectrum of neuropathological changes and α-synuclein abnormalities common to human PD[139,140], and are thus relevant for the testing of novel neuroprotective therapies. Studies in mouse and *Drosophila* models and cultured cells overexpressing wild-type or mutant α-synuclein have revealed a role for the endogenous molecular chaperone heat shock protein 70 (Hsp70) in the protection of dopamine neurons from the cytotoxic effects of α-synuclein overexpression. The naturally-occurring benzoquinone ansamycin, geldanamycin, has been shown to inhibit α-synuclein aggregation and toxicity in *Drosophila* and in cultured cells, possibly by modulating Hsp70 molecular chaperone activity, and warrants exploration as a neuroprotective strategy in PD[141]. Emerging evidence implicating the malfunction of the lysosomal degradation pathway in α-synuclein aggregation suggests that enhancing lysosomal function might also prove to be an effective neuroprotective intervention[90]. The autophagy inducer rapamycin demonstrated increased clearance of all forms of α-synuclein[89], although its long-term use is associated with many complications. However, novel modulators of autophagy might have therapeutic potential for the treatment of PD. An additional development based on the pathological involvement of α-synuclein aggregation in PD is the development

of a PD vaccine. Recently, a vaccine based on human α-synuclein elicited the generation of anti-α-synuclein antibodies in a mouse model[142]. This putative intervention offers hope to high-risk PD-mutation carriers with a positive family history of PD.

Mutations in *LRRK2* are the most common genetic determinant of PD identified to date occurring in familial and apparently sporadic PD. Pathogenic *LRRK2* mutations alter its kinase activity increased in the case of Gly2019Ser[54] and Ile2020Thr[143], which is in line with an expected gain-of-function mechanism for their dominant transmission. The increased kinase activity of mutant Lrrk2 is postulated to mediate its toxic effects in cell culture, including inclusion body formation and the triggering cell death in neurons[144]. Furthermore, kinase-dead versions of Lrrk2 are reportedly less toxic than their active equivalents, even when pathogenic mutations are present in the molecule outside of the kinase domain[145], suggesting that kinase inhibitors might offer a new therapeutic approach to treat or delay PD progression in patients with *LRRK2* mutations and perhaps in sporadic PD as well. The importance of kinase activity in disease pathogenesis remains to be tested *in vivo* in transgenic animal models with *LRRK2* mutations. Current limitations include lack of knowledge regarding substrates for Lrrk2 kinase activity and the deficiency of an available animal model.

Products of the recessive genes might also advance the development of PD therapies. The loss-of-function found in parkin-, DJ1-, and PINK1-linked PD suggests that increased expression of these proteins may prevent or ameliorate the disease in high-risk individuals and delay PD progression in carrier patients[16]. *Parkin* and *DJ1* knockout mice have been developed and might serve as important tools for the study of potential therapeutic options[146]. However, the relative rarity of *parkin*, *DJ1*, and *PINK1*-linked disease, together with their unique phenotypes, challenge their relevance to the development of treatment for sporadic PD[145].

Genetic counseling

With the identification of genes that are implicated in the causation of familial PD, the demand for genetic testing by patients and their family members as well as by physicians will surely increase. Genetic testing refers to the evaluation of an individual's genetic material (DNA) to determine the predisposition to a specific disease or to confirm the diagnosis of genetic disease. Testing of mutations in known disease-causing genes has been useful in the definition and classification of many heterogeneous inherited neurodegenerative disorders[147]. To date, molecular genetic testing is clinically

available for *parkin*, *PINK1*[148], and *LRRK2* G2019S and I2020T mutations (http://www.genetests.org), although no formal guidelines have been established by any of the international PD alliances. Whole PD gene sequencing allows the identification of alterations in the entire gene, while testing for specific known disease-causing mutations can be performed using multiple assays, such as sequence, *Taq*Man and others.

Mutation status is particularly important when results have diagnostic or therapeutic consequences. In the case of "suspected PD" in individuals with a questionable extrapyramidal clinical picture, the detection of a disease-causing gene may verify a clinical diagnosis of PD. Unfortunately, testing will not necessarily prove informative, as the failure to detect a mutation does not negate the diagnosis of PD nor does it rule out the presence of a mutation in another region, while conversely, a detected mutation might not prove pathogenic. Furthermore, a better understanding of mutation frequency, age-dependent penetrance, and natural history are necessary in order to better interpret test results and provide effective genetic counseling. Presently, in an era when PD treatment is purely symptomatic, the clinical utility of genetic testing is questionable, as mutation status will not alter the treatment of monogenic forms of PD. The decision to pursue genetic testing should be made on an individual basis and should be accompanied by genetic counseling.

While the advantages of testing asymptomatic family members include close follow up and early detection of PD as well as the opportunity to plan both psychologically and financially for the future, these must be weighed against the negative emotional and social consequences, possible employment and insurance discrimination, and inconclusive counseling due to the partial penetrance and the inadequate understanding of the age-dependant risk of the disease causing mutations. As long as there is no proven medical or behavioral treatment that can modify the natural history of PD, the justification for the clinical testing of asymptomatic family members currently remains highly questionable. The implications of genetic testing in PD are highlighted in comprehensive reviews by McInerney-Leo *et al*[149], Tan and Jankovic[147], and Klein[150].

Conclusions

In the last decade, major advances have been made in understanding the genetic basis of PD. The identification of pathogenic mutations in *PARK*-linked genes contests the once held environmental hypothesis for PD and brings the scientific community closer to the elucidation of the enig-

matic pathogenesis of this common and devastating disorder. Most importantly, understanding the molecular and cellular pathways involved will be critical for the development of desperately needed preventative, symptomatic, and curative treatment modalities.

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