Genetics of Parkinson's disease and biochemical studies of implicated gene products
Commentary

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Parkinson's disease was thought, until recently, to have little or no genetic component. This notion has changed with the identification of three genes, and the mapping of five others, that are linked to rare familial forms of the disease (FPD). The products of the identified genes, α-synuclein (PARK 1), parkin (PARK 2), and ubiquitin-C-hydrolase-L1 (PARK 5) are the subject of intense cell-biological and biochemical studies designed to elucidate the underlying mechanism of FPD pathogenesis. In addition, the complex genetics of idiopathic PD is beginning to be unraveled. Genetic information may prove to be useful in identifying new therapeutic targets and identifying the preclinical phase of PD, allowing treatment to begin sooner.

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Abbreviations

AR-JP	autosomal recessive juvenile parkinsonism
FPD	familial (monogenic) Parkinson's disease
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
PD	Parkinson's disease
PET	positron emission tomography
SPECT	single photon emission computed tomography
UCH-L1	ubiquitin-C-hydrolase-L1

Introduction

Parkinson's disease (PD) is a common (1–2% of the population over 65), age-associated neurodegenerative disease that gradually robs an individual of the ability to initiate and sustain movements and often produces a resting tremor [1]. PD involves the progressive loss of dopaminergic neurons, primarily in the substantia nigra. Although there is no clear picture of the underlying cause of neuronal loss, the symptoms of PD can be effectively, albeit transiently, treated by replacement of dopamine (via L-DOPA) or by treatment with dopamine agonists [2]. Until recently, PD was widely considered to have little or no genetic component because a long preclinical phase makes a family history difficult to discern; >50–60% of the nigral neurons can be lost with no obvious clinical consequence. Thus, concordance between siblings appeared to be insignificant when PD was defined solely by clinical criteria. However, the emergence of improved PET and SPECT imaging methods has allowed the number of dopaminergic neurons in the substantia nigra to be estimated [2], and a significant concordance has been revealed. In the near future, provided that genetic susceptibility factors for PD can be identified, one can imagine using imaging to diagnose preclinical PD in high-risk populations, allowing treatment to begin before symptoms are apparent.

Our understanding of the complex genetics of PD is based on seven monogenic familial forms (Table 1). This review discusses experimental papers published in 2001 that involve the genetics and clinical features of each of these forms and, where applicable, biochemical and cell-biological studies.

PARK1 (α-synuclein)

The A53T mutation of the α-synuclein gene [3] is rare but has been found in several kindreds living in, or originating from, Greece [4,5,6•]. Family members bearing the A53T mutation have an early mean age at onset and a short mean disease duration [6•]. PD in most of these patients is akineto-rigid (association of slowing of movement and increased tone) and tremor is significantly less frequent than in idiopathic PD (referring to all forms of PD that are not monogenic). Other features that distinguish these families from idiopathic PD are cognitive decline [4,6•], severe central hypoventilation, orthostatic hypotension, myoclonus, and urinary incontinence. In one family, two autopsied cases revealed that, in addition to the cell loss that is typical of idiopathic PD, there were neuritic pathological changes in the deeper cortical layers and marked gliosis, predominantly in the basal ganglia [6•]. This distribution of pathology was consistent with the clinical features, which overlap with multiple system atrophy and dementia with Lewy bodies. In addition to the A53T mutation, the A30P mutation was identified in one German family [7].

Although the centrality of α-synuclein to PD pathogenesis is supported by pathological and biochemical studies, genetic studies attempting to link polymorphisms in this gene to idiopathic PD are inconclusive. Two recent association studies found no association between a repeat in the α-synuclein gene, or the combination of this repeat and the apolipoprotein E genotype, and PD [8,9]. However, a third study identified 10 new single nucleotide polymorphisms in the α-synuclein gene and found an association between one of these polymorphisms and PD [10]. These results suggest that the magnitude of increased susceptibility as a result of polymorphisms in the α-synuclein gene is likely to be small and population-dependent.

Although α-synuclein is highly expressed in brain, its function is unknown. It is the primary fibrillar component

Table 1

Main characteristics of monogenic forms of parkinsonism.

Designation	Locus	Gene	Transmission	Mean age at onset (years)	Progression	Clinical features	Lewy bodies
PARK1	4q21–22	α-synuclein	AD	≈ 45 (20–85)	Rapid	Frequent atypical features: dementia, central hypoventilation, myoclonus, abnormal eye movements, urinary incontinence	+
PARK2	6q25–27	Parkin	AR	Early (3–64)	Very slow	Frequent features: dystonia at onset, brisk reflexes, sleep benefit, very good response to levodopa Rare: dementia	– (Except one case)
PARK3	2p13	?	AD	59 (37–89)	Slow	Reduced penetrance (≈ 40%); frequent dementia associated with neurofibrillary tangles and senile plaques	+
PARK4	4p15	?	AD	33	Rapid	Frequent atypical features: early weight loss, dysautonomia and dementia. Several haplotype carriers present with postural tremor only	+
PARK5	4p14	UCH-L1	Probable AD	50	?	Typical PD. Reduced penetrance.	ND
PARK6	1p35–36	?	AR	≈ 40 (30–68)	Slow	Similar to parkin cases.	ND
PARK7	1p36	?	AR	≈ 33 (27–40)	Slow	Similar to parkin case but frequent behavioral disturbances and focal dystonia.	ND
PARK8	12p11.2–q13.1	?	AD	51	?	Reduced penetrance	–

AD, autosomal dominant; AR, autosomal recessive; ND, not determined.

of Lewy bodies, the neuronal inclusions that characterize the PD substantia nigra. The A53T and A30P mutations predispose the protein to *in vitro* oligomerization [11,12] and the overexpression of α-synuclein in either mice or *Drosophila* produces features of PD. These results converge with the genetic evidence to suggest that a gain of α-synuclein toxic function, linked to its fibrillization, is responsible for PD pathogenesis (see Figure 1). However, it is possible that loss of normal function is partly responsible for PD.

Several biophysical studies of the intrinsic structural properties of α-synuclein and its oligomerization/fibrillization have appeared [12,13,14•,15•,16]. These confirm that α-synuclein exists in dilute solution as an ensemble of conformations, but that mutations increase the population of β-sheet-containing, possibly prefibrillar, conformers [14•]. Several studies have demonstrated that environmental factors implicated in PD, including heavy metals [17] and pesticides [18], accelerate *in vitro* fibril formation. However, the relevance of these studies to PD has yet to be demonstrated, as the PD-promoting A30P mutation also decreases the rate of *in vitro* fibril formation (while increasing formation of prefibrillar oligomers, or protofibrils) [11,12].

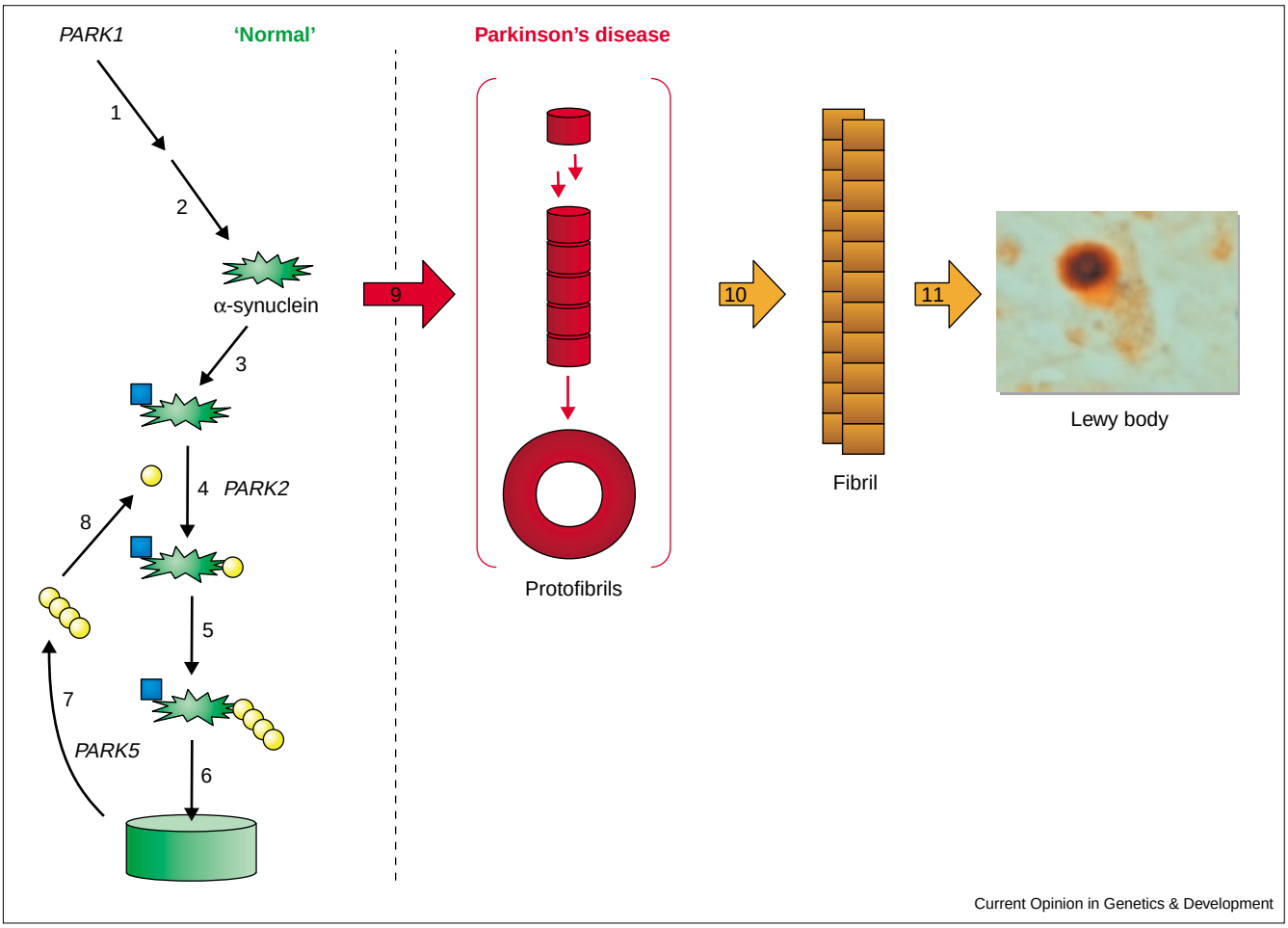
Several studies involving the interaction of α-synuclein with lipids, both *ex vivo* [19•,20,21], and *in vitro* [22,23] have appeared. Some evidence suggests that lipid binding may influence the oligomerization/fibrillization pathway. In addition to the helix-based monomer–lipid interaction, a second mode of interaction with lipids, involving β-sheet rich protofibrils, was characterized [15•].

Several examples of the post-translational modification of α-synuclein have been reported. A glycosylated form of α-synuclein was isolated from PD brain and demonstrated to be a substrate for the E3 ubiquityl ligase parkin (Figure 1, step 4) [24••]. Tyrosine phosphorylation was demonstrated to occur in cell culture) [25,26] and tyrosine nitration was shown to be induced by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treatment of mice [27]. Finally, the covalent modification of α-synuclein by the ortho-quinone derived from oxidation of dopamine was shown to stabilize protofibrils and inhibit their *in vitro* conversion to fibrils (Figure 1, step 10) [28••]. No genetic evidence to support the importance of any of these posttranslational modifications in PD (e.g. mutations in antioxidant proteins or kinases) has been reported.

β-synuclein has not been directly linked to PD, but the extent and location of α- and β-synuclein mRNA expression in PD brain suggests that the latter may modify the pathogenicity of the former [29]. Strikingly, the Parkinsonian phenotype of the α-synuclein transgenic mouse was virtually eliminated by crossing with a β-synuclein-expressing mouse [30]. Other proteins, including tubulin [31], synphilin [32], and the dopamine transporter [33] were shown to interact with α-synuclein, but genetic evidence for their involvement in PD is lacking [34,35]. The one interacting protein that has been linked to PD is parkin, which interacts with O-glycosylated α-synuclein (Figure 1, step 4) [29]. O-glycosylation of synaptosomal proteins effects many synaptosomal proteins, including β-synuclein and UCH-L1 [36•].

Because fibrillization is highly concentration-dependent, factors that promote α-synuclein expression (e.g. NGF [37]) could increase susceptibility to idiopathic PD (Figure 1, steps 1 and 2) [10]. Studies of its expression will be aided by the mapping of the α-synuclein promoter and the development of a cell-based assay in which it drives

Figure 1



A model of how the three characterized PD genes, *PARK1*, *PARK2*, and *PARK5*, may converge to promote Parkinson's disease. All of the indicated steps (1–11) could be either promoted or inhibited by endogenous factors that may effect PD susceptibility. Under 'normal' circumstances, the cytoplasmic concentration of α-synuclein should be tightly controlled, in order to prevent its highly concentration-dependent conversion to protofibrils, then fibrils. For example, the transcription (step 1) and translation (2) of PARK1 may be upregulated in PD, resulting in abnormally high cytoplasmic concentration of α-synuclein. Thus, the degradation of cytoplasmic α-synuclein is also critical. Glycoslyation (blue square) of α-synuclein (3) may be required for its subsequent ubiquitylation by parkin, the *PARK2* product (4 and 5). Proteasomal degradation of ubiquitylated α-synuclein (6) would produce peptide–ubiquitin conjugates, and subsequent recycling (7 and 8) of ubiquitin (yellow circles) may be controlled by the *PARK5* product, UCH-L1. If problems arise in steps 1–8, or if mutations in *PARK1* promote oligomerization, α-synuclein will form structured protofibrils (step 9), which may be the pathogenic species in PD (protofibrils are morphologically heterogeneous; spheres, chains, and rings have been identified). These intermediates are eventually converted to fibrils (10), then to Lewy bodies (11), which are the pathological hallmark of the PD brain. Endogenous factors that inhibit the formation of protofibrils (step 9) could protect against PD, whereas those that inhibit conversion of protofibrils to fibrils (step 10) may promote PD.

luciferase expression [38]. Mitochondrial inhibitors (MPTP and rotenone) induce parkinsonism in animals and promote α-synuclein inclusion formation [39]. Proteasome inhibitors also induce inclusions (Figure 1, step 6) [40]. The relationship between proteasome activity and α-synuclein expression [41] is especially interesting in light of the fact that proteasome activity is reduced in PD brain [42] and that PARK2 and PARK5 encode components of the proteasomal degradation pathway (see below and Figure 1).

PARK2 (parkin)

PARK2 constitutes an important locus for autosomal recessive PD and isolated early-onset cases [43], including one case where age at onset was 64 [44]. No pathogenic parkin mutations were identified in 95 isolated cases and 23 cases with probable autosomal recessive PD with onset after the age of 45 [45]. These preliminary data suggest that parkin mutations are rare among patients with late onset PD, but sequencing of the parkin-coding exons was only performed in a subset of patients and heterozygous exon rearrangements were not tested in this study. The spectrum of parkin mutations now includes at least 60 different mutations. In addition to the frequent point mutations and exon rearrangements, which call for a combination of sequencing and quantitative PCR for mutation screening [46], the first splicing mutation has been

identified and functionally validated (A Brice, unpublished data). The first missense mutation (R42P) in the ubiquitin-like domain of parkin has been identified [47]. In addition, the promoter region of the gene has been sequenced in patients in whom only a single heterozygous parkin mutation was detected. Several polymorphisms were characterized but none were causative [48]. Among parkin mutations, several are found more than once in different populations. Using intragenic and closely flanking markers, Periquet *et al.* [49•] demonstrated that exon rearrangements occurred independently, whereas several point mutations — including the c.255delA and Arg275Trp mutations — most probably result from a founder effect, ancient enough to account for their spread to several European countries.

The clinical spectrum associated with parkin mutations is expanding [43], with some slowly progressing cases [50] and others with cerebellar signs [51–53]. PET studies in parkin cases [54] confirm that parkin patients show a marked reduction of fluoro-dopa uptake, not only in the putamen, as in idiopathic PD, but also in the caudate nucleus. In addition, dopamine D2 receptor binding, measured with ^{11}C-raclopride, was reduced in the caudate nucleus, unlike in idiopathic PD. Interestingly, significantly reduced fluorodopa uptake was found in unaffected parkin carriers compared to controls, suggesting an infraclinical involvement of the nigro-striatal dopaminergic pathway [54]. If confirmed, these results would suggest that the nigro-striatal dopaminergic pathway is impaired in parkin heterozygotes. Two recent post-mortem studies of parkin mutants [52,55•] provide conflicting results concerning the anatomic distribution of Lewy bodies and neuronal loss. A 75-year-old parkin patient showed a selective loss of dopaminergic neurons in the substantia nigra pars compacta without Lewy bodies but, surprisingly, with degeneration of parts of the spinocerebellar system [52]. However, a 52-year-old compound heterozygote for parkin mutations (R275W and deletion of 40 bp in exon 3), had a marked loss of dopaminergic neurons in the substantia nigra but with numerous Lewy bodies, which were also found in the locus ceruleus and the nucleus basalis of Meynert [55•]. In the latter case, as well as in another family with the 40-bp deletion of exon 3, PD was observed in successive generations, suggesting autosomal dominant transmission. This hypothesis is supported by the finding of presynaptic dopaminergic dysfunction in asymptomatic parkin heterozygotes [53]. However, pseudo-dominant inheritance, as already described [44,56–58], could also account for this observation. It is interesting that, despite the lack of parkin function, Lewy bodies were observed in a parkin case [59], suggesting that ubiquitylation is not required for Lewy body formation (Figure 1).

The parkin protein is an E3 ubiquityl ligase, involved (along with a cognate E2 protein) in the attachment of ubiquitin to proteins that are targeted for proteasomal degradation. E3 ligases often demonstrate some substrate selectivity. Several studies that appeared in the past year have identified substrates of parkin. A putative transmembrane G-protein-coupled protein Pael receptor, which causes unfolded protein response-induced cell death when overexpressed, accumulates in an insoluble form in AR-JP brain [60••]. Co-expression of parkin (but not the truncated form) protects against cell death [61]. Parkin also promotes the proteasomal degradation of itself and of CDCrel-1, a synaptic vesicle protein [62]. Finally, parkin has been shown to promote degradation of α-synuclein, via an O-glycosylated form [24••]. The possibility that parkin is responsible for the degradation of α-synuclein suggests that parkin may work to indirectly suppress α-synuclein oligomerization/fibrillization, by lowering the cytoplasmic concentration of α-synuclein (Figure 1). One study localized parkin to lipid rafts and postsynaptic densities and showed that the autosomal recessive PD truncation eliminated the localization [63].

PARK5 (UCH-L1)

A point mutation in the gene encoding ubiquitin C-hydrolase-L1, I93M, was identified in two siblings from a family in which PD was apparently dominantly transmitted [64]. However, as neither of their parents had been diagnosed with PD, this mutation may not be 100% penetrant. The I93M mutation has not been identified in any other individuals, whereas a common polymorphism (S18Y) has [65–67]. Three of these studies have demonstrated that Y18 is associated with decreased risk of PD and that the effect is dose-dependent [65–67].

UCH-L1 is a highly expressed protein in the brain, constituting possibly 1% of brain protein. Its function is unknown, though it is presumed to act to recycle ubiquitin by hydrolyzing the ubiquitylated peptides, the products of the proteasome (Figure 1, step 7). UCH-L1 is capable of cleaving ubiquitin carboxy-terminal amides *in vitro* and the I93M mutation reduces this activity by ~50% [64]. The effect of the S18Y polymorphism on this activity is unknown.

PARK6

This locus was recently mapped to chromosome 1p35–p36 in a Sicilian family with AR early-onset parkinsonism [68•]. Subsequently, suggestive evidence of linkage was demonstrated in 10 other families, from Italy, the Netherlands, Great Britain and Germany, allowing the candidate region to be restricted to a 9cM interval [69,70]. Age at onset ranged between 30 and 68 years, indicating the existence of late-onset cases. The main features are slow progression, good and persistent response to levodopa but with frequent drug-induced dyskinesias very similar to the parkin cases from Europe reported to date.

PARK7

PARK7 was mapped to chromosome 1p36 in patients with AR early-onset parkinsonism living in an isolated community in the south-west of the Netherlands [71•]. The 16cM candidate interval is located 25cM telomeric to PARK6,

excluding the possibility of allelism. Confirmation of linkage was obtained in two additional pedigrees from Italy and the Netherlands [72]. Age at onset ranged from 27–40 years and the phenotype was similar to parkin disease and PARK6-linked families. However, behavioral disturbances and focal dystonia appeared to be frequent in PARK7 patients.

PARK8

Linkage analysis was performed in a large Japanese family with autosomal dominant parkinsonism, which included fifteen patients [73•]. After exclusion of known loci, PARK8 was mapped to a 13.6 cM interval on chromosome 12p11.2-q13p.1. All patients had dopa-responsive parkinsonism, with a mean age at onset of 51±6 years. The haplotype segregating the disease was detected in five unaffected carriers, two of whom were older than the average at age at onset. This result suggests PARK8 might be associated with reduced penetrance. Interestingly, neuropathological examination in four cases revealed pure nigral degeneration without Lewy bodies. Since no other families have been tested for linkage to PARK8, its relative frequency is still unknown.

Genome-wide scan in PD families

A new era of PD research has been opened by the reports of genome-wide screens performed in two independent PD data sets [74,75]. The first comprised 113 PD-affected sibling pairs and detected 4 regions with suggestive evidence for linkage with maximum likelihood scores ranging from 0.93 to 1.30 on chromosomes 1, 9, 10 and 16 [74]. Although no evidence of linkage was observed in regions corresponding to the genes involved in familial PD, chromosome 9q33–q34.1 contains the gene encoding torsin A, responsible for early-onset torsion dystonia, and the dopamine β-hydroxylase gene. The second data set comprised 174 families with multiple PD individuals, including 185 affected sibpairs [75]. Three regions on chromosomes 5q, 8p and 17q generated multipoint lod scores suggestive of linkage ranging from 1.5 to 2.22. After stratification according to age at onset or dopa responsiveness, other potential candidate regions were revealed. Not surprisingly, the 18 families with early-onset generated a significant multipoint lod score of 5.47 for a marker located in the parkin gene, in which mutations were identified in 11/18 kindreds. Lod scores suggestive of linkage were also found for two regions on chromosomes 3q and 9q in the 9 families with no response to levodopa. The observation of suggestive linkage to a region of chromosome 17 located close to the *tau* gene both in the whole group and in late-onset families raises the question of a possible connection between PD and tau. Of note, three recent large case-control studies established a significant association between polymorphisms of the *tau* gene and PD [76–78]. It is widely recognized that the *tau* gene is a genetic risk factor for progressive supranuclear palsy and corticobasal degeneration [79], which are characterized by the presence of tau deposits in the brain, but how tau could influence α-synuclein pathology in PD remains to be elucidated. Except for the confirmation of the importance of the parkin locus in early-onset PD, no significant linkage was established in either of the genome-wide studies, even in regions involved in other monogenic forms of PD, and not much overlap was found between regions presenting suggestive evidence for linkage. This result indicates that, as with many complex diseases, no major genetic factor has yet been identified that would support the hypothesis of the interaction of several genetic and environmental factors, each responsible for a small effect. Nevertheless, a susceptibility locus for late onset PD in Iceland is about to be reported (see [80]).

Conclusion

Genetic approaches to PD have been very fruitful in identifying loci and genes involved in monogenic forms of the disease, leading to major advances in our understanding of the physiopathology of this disorder. Attempts to map or identify genetic risk factors in PD have been less successful, despite several large-scale projects. No doubt this goal will be achieved in the future by combining the analysis of larger series of families with the use of new tools derived from the Human Genome Project in order to develop strategies for rational preventive or curative treatment of PD.

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