

Genetic clues to the pathogenesis of Parkinson's disease

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Recent years have seen an explosion in the rate of discovery of genetic defects linked to Parkinson's disease. These breakthroughs have not provided a direct explanation for the disease process. Nevertheless, they have helped transform Parkinson's disease research by providing tangible clues to the neurobiology of the disorder.

Parkinson's disease (PD) is the second most common human neurodegenerative disorder, after Alzheimer's dementia. This disease is progressive, with a mean age at onset of 55, and its incidence increases markedly with age¹. The primary hallmark of PD is the degeneration of the nigrostriatal dopaminergic pathway, which, in depleting the brain of dopamine, initiates aberrant motor activity such as tremor at rest, rigidity, slowness of voluntary movement, and postural instability. As with other neurodegenerative disorders, however, the neuropathology of PD is not restricted to one pathway, and histological abnormalities also occur in many other dopaminergic and non-dopaminergic cell groups including the locus coeruleus, raphe nuclei and nucleus basalis of Meynert². Because numerous distinct neurological conditions share the clinical features of PD, a definitive diagnosis of PD can only be made at autopsy, and it has customarily been based not only on the loss of nigrostriatal dopaminergic neurons but also on the presence of intraneuronal inclusions called Lewy bodies (LBs). These are spherical eosinophilic cytoplasmic aggregates containing a variety of proteins, of which α -synuclein is a major component³, and are found in every affected brain region. Whether identification of LBs should still be considered necessary for the diagnosis of PD is controversial, in that individuals with inherited PD linked to mutations in the gene encoding parkin typically lack LBs and are still regarded as having PD¹. Moreover, the role of LBs in the PD neurodegenerative process is a matter of fierce debate.

The cause of almost all cases of PD remains unknown. PD generally arises as a sporadic condition but is occasionally inherited as a simple mendelian trait (Table 1). Although sporadic and familial PD are very similar, inherited forms of the disease usually begin at earlier ages and are associated with atypical clinical features (Table 1). Until recently, all of the hypotheses regarding the cause and mechanism of PD neurodegeneration derived from investigations carried out on autopsy tissues from individuals with sporadic PD or in neurotoxic animal

models such as that produced by the mitochondrial poison 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)¹. In the mid-1990s, however, this situation changed with the identification of a mutation in the α -synuclein gene associated with PD in an Italian kindred⁴. Since then, four additional genetic defects underlying PD have been identified and linkages have been reported for at least four more (Table 1).

Here we review what is currently known about these PD-causing mutations. As is the case in Alzheimer's disease, these gene defects seem to operate on a common molecular pathway. Thus, we also discuss this pathway and the directions in which those genes may lead us in regard to the development of genetically based animal models, which are crucial to unraveling the basis of the neurodegenerative processes of PD.

α -Synuclein mutations and dopaminergic neurodegeneration

Three missense mutations (A53T, A30P and E46K) in the gene encoding α -synuclein are linked to a dominantly inherited PD⁴⁻⁶ (Table 1). None of these mutations has been found in sporadic PD or in individuals without the disease.

Injection of either human wild-type or mutant α -synuclein-expressing viral vectors into the rat and monkey nigrostriatal pathway causes dopaminergic neurodegeneration associated with α -synuclein-containing inclusions^{7,8}. Transgenic overexpression of mutant or wild-type α -synuclein in mice or flies has produced equivocal results¹, however, in that intraneuronal proteinaceous inclusions, but not definite neuronal death, have generally been documented. Still, these results, together with the finding that α -synuclein ablation in mice does not cause neurodegeneration^{9,10}, support the notion that α -synuclein mutations operate by a toxic gain-of-function mechanism. Viral vector-mediated overexpression of wild-type α -synuclein reproduces PD neuropathology in animals^{7,8}, and genomic multiplication of the gene encoding α -synuclein is associated with a familial form of PD^{11,12}. It is thus possible that the function gained by the mutant protein is not a newly acquired property, but rather a native property that is enhanced and becomes deleterious.

How mutant α -synuclein variants produce neurotoxicity remains elusive, in part because the protein's function is just beginning to be understood. Wild-type α -synuclein binds preferentially to plasma membranes (rather than mitochondrial membranes) in yeasts¹³ and this interaction, which is mediated by major conformational changes of the protein¹⁴, seems to be crucial to several of its physiological functions¹. Membrane-bound α -synuclein has been proposed to modulate phospholipase D activity¹⁵, thereby perhaps influencing the availability of synaptic vesicles for release. Membrane-bound α -synu-

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Table 1 Genes and loci linked to familial PD

Locus	Chromosomal location	Protein	Inheritance pattern	Atypical PD features	Lewy bodies
<i>PARK1</i>	4q21	α -Synuclein ^a	AD	Early onset Lower prevalence of tremor	Yes
<i>PARK2</i>	6q25.2–q27	Parkin	AR	Early or juvenile onset More frequent dystonia and levodopa-induced dyskinesias Slower disease progression	Mostly negative ^b
<i>PARK3</i>	2p13	Unknown	AD	Dementia in some individuals Rapid progression	Yes
<i>PARK4^c</i>	4p15	Unknown	AD	Early onset Rapid progression Dementia Autonomic dysfunction Postural tremor	Yes
<i>PARK5</i>	4p14	UCH-L1	AD	None	Unknown
<i>PARK6</i>	1p36	PINK1	AR	Early onset Slow progression	Unknown
<i>PARK7</i>	1p36	DJ-1	AR	Early onset Psychiatric symptoms Slow progression	Unknown
<i>PARK8</i>	12p11.2–q13.1	Unknown	AD	None	No
<i>PARK9</i>	1p36	Unknown	AR	Juvenile onset Spasticity Supranuclear gaze paralysis Dementia	Unknown

AD, autosomal dominant; AR, autosomal recessive. ^aIncluding mutations and wild-type multiplications. ^bLewy bodies reported in one individual with parkin mutations⁵⁴. ^cThe initial PARK4 linkage to 4p15 could not be confirmed, and the PD phenotype in this family was subsequently linked to a PARK1 variant (α -synuclein triplication)¹¹.

clein also seems to be in dynamic equilibrium with cytosolic α -synuclein¹³, which upon accumulation can render endogenous dopamine toxic¹⁶, and to act as a seed promoting the formation of cytosolic inclusions¹⁷. Conceivably if these aggregates are not promptly cleared by degradation pathways¹⁸, neurotoxicity can ensue.

Both wild-type and mutant α -synuclein form amyloid fibrils akin to those seen in LBs, as well as nonfibrillary oligomers¹ termed protofibrils. Because the pathogenic α -synuclein^{A53T} mutant promotes the formation of protofibrils¹⁹, these oligomers may be the toxic species of α -synuclein. In keeping with this and with the known association of α -synuclein with synaptosomes, protofibrils may cause toxicity by permeabilizing synaptic vesicles²⁰, allowing dopamine to leak into the cytoplasm and participate in reactions that generate oxidative stress. Furthermore, the selective vulnerability of nigrostriatal neurons in PD may derive from the ability of dopamine or dopamine-quinone to stabilize α -synuclein protofibrils²¹. However, protofibrils have only been identified and studied *in vitro*, and so further work is required to establish whether they form in neurons and whether their formation correlates with neurotoxicity.

Parkin, a protein with many substrates

Loss-of-function mutations in the gene encoding parkin cause a recessively inherited form of PD²² (Table 1). The onset of parkin-related PD usually, but not always, occurs before age 30 (ref. 1). Pathologically, this form of familial PD is associated with a loss of nigrostriatal neurons, but LBs are not typically observed. Parkin-null mice and flies do not develop degeneration of nigrostriatal dopaminergic neurons^{23–25}. However, these animals do show functional mitochondrial deficits^{24–26} suggestive of those seen in sporadic PD¹.

Identification of the normal function of parkin has provided hints to the pathogenic effects of parkin mutations. Parkin is one of a class of proteins containing two RING-FINGER DOMAINS separated by an in-between RING-finger domain, and like other such proteins, parkin functions as an E3 ubiquitin ligase^{27,28}, a component of the ubiquitin

system. Many mutations affecting parkin abolish its E3 ligase activity¹, as does the post-translational modification (*S*-nitrosylation) of wild-type parkin²⁹. It is thus conceivable that parkin dysfunction is involved in the pathogenesis of both familial and sporadic PD, but the underlying molecular details remain speculative. Loss of parkin activity may trigger cell death by rendering neurons more susceptible to cytotoxic insults, such as those caused by proteasome inhibition or mutant α -synuclein³⁰, or by impairing ubiquitination of cyclin E³¹, a molecule previously implicated in neuronal apoptosis. In support of the latter hypothesis, cyclin E is abundant in the midbrains of individuals with parkin-related PD, and overexpression of wild-type parkin attenuates cyclin E accumulation and promotes survival in excitotoxin-treated cultured neurons³¹. Several studies have shown functional interactions between parkin and α -synuclein, and have suggested that these interactions may involve the proteasome¹. Other investigations have highlighted the multiplicity of parkin substrates and how these might have a key role in neuronal death¹. However, none of the parkin substrates that have been identified seem to be specifically enriched in dopaminergic neurons. Thus, further studies are needed to explain the relative specificity of dopaminergic neurodegeneration mediated by parkin mutations.

UCH-L1 dabbles in degeneration

Ubiquitin C-terminal hydrolase-L1 (UCH-L1) is expressed mainly in the brain, where it catalyzes the hydrolysis of C-terminal ubiquitin esters. A single dominant mutant form (I93M) of UCH-L1, found in two members of a PD-affected family, has been implicated in the development of an inherited form of PD³². Conversely, it has been confirmed that a polymorphism (S18Y) of UCH-L1 reduces the risk of developing sporadic PD, especially in early-onset cases³³. The I93M mutation decreases the enzyme's activity, suggesting that a loss of function is the culprit in disease development. However, mice carrying a UCH-L1-null mutation do show neurodegenerative changes, but not in the nigrostriatal dopaminergic pathway³⁴. Upon

GLOSSARY

RING domain One of a class of protein domains that consist of two loops that are held together at their base by cysteine and histidine residues that complex two zinc ions. Proteins containing domains of this type are known as RING-finger proteins.

Sumoylation The attachment of SUMO, a ubiquitin-like modifier protein. But in contrast to ubiquitin, which targets proteins for degradation, SUMO seems to affect the subcellular localization of proteins and enhance their stability.

Non-cell-autonomous A genetic trait in which genotypically mutant cells cause other cells (regardless of their genotype) to show a mutant phenotype. By contrast, a cell-autonomous trait is one in which only genotypically mutant cells show the mutant phenotype.

Autozygosity Homozygosity by virtue of parental descent from a common ancestor.

through a loss of function. Yet reduced striatal [¹⁸F]dopa uptake revealed by positron emission tomography has been found in asymptomatic *PARK6* heterozygotes, raising the possibility that *PINK1* mutations may operate through haploinsufficiency or a dominant-negative effect⁵³. In either case, impaired phosphorylation of *PINK1*'s substrate, especially in mitochondria, is a likely scenario for the pathogenic mechanism of the two mutations.

Although the crucial substrates of *PINK1* have yet to be identified, we already know that neuroblastoma cells transiently transfected with either wild-type or mutant *PINK1* do not show detectable alterations in viability⁵⁰. In contrast, when these cells are challenged with the proteasome inhibitor MG132, overexpression of wild-type *PINK1* mitigates cell death, whereas overexpression of mutant *PINK1* neither attenuates nor enhances MG132-mediated cytotoxicity⁵⁰. These results suggest that the loss of *PINK1* function renders dopaminergic neurons more vulnerable to injury. This possibility does fit neatly into the concept that interactions between genetic and environmental factors may be responsible for the neurodegeneration in sporadic PD¹.

Conclusion

The shared phenotype associated with the different genetic mutations we have discussed raises the tantalizing possibility of a molecular intersection in the pathogenic mechanisms driven by these distinct PD-causing mutations (Fig. 1). Among various plausible mechanistic hypotheses, available data favor impaired protein degradation and accumulation of misfolded proteins as the unifying factor linking genetic alterations to dopaminergic neurodegeneration in familial PD (Fig. 1). According to this reasoning, α -synuclein and DJ-1 mutations would cause abnormal protein conformations, overwhelming the main cellular protein degradation systems—the proteasomal and lysosomal pathways—whereas parkin and UCH-L1 mutations would undermine the cell's ability to detect and degrade misfolded proteins. The common end result of these different perturbations is thus expected to be a cellular buildup of unwanted proteins that should have been cleared. Minimal defects in this protein turnover machinery may suffice to cause a slow demise of dopaminergic neurons, which may explain the relentless, progressive nature of the disease. This scenario does not, however, explain why an accumulation of misfolded proteins, which is likely to occur in all cells, would inflict

greater damage on dopaminergic neurons in familial PD. Perhaps nigrostriatal dopaminergic neurons are less able to cope with 'misfolded protein stress' because of a higher basal load of damaged proteins that is due to dopamine-mediated oxidative events. Also poorly addressed by the above scenario is the link between previously identified factors in PD neurodegeneration, such as mitochondrial dysfunction or oxidative stress, and the molecular events engendered by the PD-causing mutations. The hypothesized mitochondrial location of DJ-1 and *PINK1* and the role of DJ-1 in oxidative stress may emerge as crucial in efforts to reconcile the different aspects of the unified pathogenic cascade of PD.

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