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Protein Kinases Linked to the Pathogenesis of Parkinson's Disease

Two papers (by Paisán-Ruíz et al. and by Zimprich et al.) in this issue of *Neuron* identify a causative gene, *LRRK2*, for familial parkinsonism. Several dominantly inherited missense mutations have been identified in a number of families that exhibit a broad spectrum of neuropathological features, including deposition of α-synuclein and tau proteins. The *LRRK2* gene is predicted to encode a large protein containing leucine-rich repeats and Ras/GTPase, tyrosine kinase-like, and WD40 domains.

Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting up to 50% of individuals over age 85. PD is characterized by the clinical triad of resting tremor, rigidity, and bradykinesia, which typically responds to levodopa therapy. The neuropathologic hallmarks of PD are loss of dopaminergic neurons and deposition of cytoplasmic protein aggregates termed Lewy bodies in the substantia nigra. The movement disorder in PD is thought to arise from reduced dopaminergic input to the striatum as a result of nigral degeneration. Parkinsonism, a clinical syndrome with features resembling those of PD, is also characteristic of a variety of distinct neurologic disorders, such as Lewy body dementia (LBD), multisystem atrophy (MSA), and progressive supranuclear palsy (PSP). These disorders are distinguished from PD by the presence of additional clinical and neuropathological features, such as the dementia and cerebral cortical Lewy bodies in LBD.

Research on the pathogenesis of PD has been spurred in recent years by the identification of several genes responsible for familial forms of parkinsonism (Gwinn-Hardy, 2002). Missense and triplication mutations in the *α-synuclein* gene cause autosomal-dominant (AD) familial parkinsonism, whereas loss-of-function mutations (large deletions and truncations as well as missense mutations) in the *parkin*, *DJ-1*, and *PINK1* genes cause autosomal-recessive (AR) inheritance of parkinsonian syndromes (Table 1). The identification of pathogenic mutations in α-synuclein, a major constituent of Lewy bodies, provides a direct link to the neuropathology of PD and related disorders, suggesting that α-synuclein deposition is relevant to the degeneration of nigral neurons. While the clinical and neuropathological spectrum in kindreds bearing α-synuclein mutations includes typical PD and LBD, parkin-linked cases generally exhibit selective loss of nigral neurons in the absence of Lewy bodies, even though the clinical features of patients bearing parkin mutations are often indistinguishable from idiopathic PD. Parkin can function as an E3 ubiquitin ligase, suggesting that altered protein turnover may promote nigral degeneration independent of α-synuclein aggregation. The association of loss-of-function mutations in parkin, DJ-1, and PINK1 with familial parkinsonism and nigral degeneration indicates that these gene products are essential for the survival of dopaminergic nigral neurons. The mechanism by which loss-of-function mutations in each of these recessive parkinsonian genes causes nigral degeneration is the focus of intense research on the pathogenesis of parkinsonism (Shen and Cookson, 2004). As yet, there are few clues to the normal function of PINK1 and DJ-1; PINK1 contains a mitochondrial targeting motif and a protein kinase domain that is highly homologous to the serine/threonine kinases of the calcium/calmodulin family, while DJ-1 lacks known functional domains.

In this issue of *Neuron*, two new studies identify a novel gene on chromosome 12 as the PARK8 locus previously linked to familial parkinsonism (Paisán-Ruíz et al., 2004; Zimprich et al., 2004). In the report by Paisán-Ruíz et al., two different missense mutations in the *PARK8* gene (which was renamed after online publication *LRRK2* [*leucine-rich repeat kinase 2*]) segregated with disease in four Basque families (R1396G) and a fifth large family from the United Kingdom (Y1654C). Additional examination of 231 PD patients from Spain and North America, some of whom had a positive family history of PD, yielded 11 Spanish cases carrying the R1396G mutation. Zimprich et al. identified missense mutations in the same two amino acid residues of the *LRRK2* gene in two large pedigrees of German-Canadian and probable English (residing in western Nebraska) origin. In the former case, the identified mutation leads to an identical missense mutation (Y1699C, with the different numbering owing to the inclusion of an additional 45 amino acids corresponding to exon 6), while in the latter case a distinct mutation leads to a different substitution in the same arginine residue (R1441C). Analysis of an additional 32 families led to the identification of the R1441C mutation in an unrelated family, as well as the identification of two further missense mutations (I1122V, I2020T) and a putative splice site mutation in three other families. In both studies, the identified mutations segregated with disease and were highly penetrant. The absence of the identified nucleotide substitutions in $>$1000 control chromosomes in each study further argues in favor of pathogenic mutations rather than polymorphisms. It will be interesting to find out whether the Japanese Sagamihara kindred, in which the PARK8 linkage was initially identified (Funayama et al., 2002), carries one of these identified mutations or a distinct mutation.

The predicted product of the *LRRK2* gene is a large protein, LRRK2/dardarin (2527 amino acids encoded by 51 exons). LRRK2 and the related predicted protein LRRK1 were initially identified on the basis of genomic and EST information as members of a novel family of the tyrosine kinase-like group of protein kinases, which bear sequence similarity to both tyrosine and serine/threonine kinases (Manning et al., 2002). The LRRK2 coding sequence comprises multiple conserved domains: 12 leucine-rich repeats, a Ras/small GTPase su-

Table 1. Familial Parkinsonism

	Locus	Gene	Inheritance
PARK1	4q21-22	*α-synuclein*	AD
PARK2	6q25-27	*parkin*	AR
PARK3	2p13	?	AD
PARK4	4p15	?	AD
PARK5	4p14	*UCH-L1*	AD
PARK6	1p35-36	*PINK1*	AR
PARK7	1p36	*DJ-1*	AR
PARK8	12p11.2-q13.1	*LRRK2*	AD

perfamily domain, a nonreceptor tyrosine kinase-like domain, and a WD40 domain. This domain architecture has been proposed to define a family of multifunctional Ras/GTPases, termed Roc (for Ras of complex proteins) (Bosgraaf and Van Haastert, 2003). The presence of LRR and WD40 motifs suggests that protein-protein interactions play an important role in the function of LRRK2, which may function as a component of a multiprotein complex. The Ras-like small GTPases (e.g., Ras, Rap1, Rho/Rac, Rab, and Ran) act as molecular switches regulating diverse cellular processes, including mitogenic signaling, cytoskeletal reorganization, vesicle trafficking, and nucleocytoplasmic transport (Takai et al., 2001). Interestingly, the tyrosine kinase-like MAPKKKs are primary effectors of small GTPases; the presence of similar domains in LRRK2 raises the possibility that its kinase activity may undergo GTP-dependent autoactivation. Characterization of the predicted GTPase and kinase activities, their regulation, and the interaction of LRKK2 with specific binding proteins and substrates will likely provide clues to its role in dopaminergic neuronal function and survival. Importantly, the identified mutations in LRRK2 all affect highly conserved residues in multiple domains, and in particular, the identification of two distinct mutations in the same amino acid (R1441G, R1441C) within the GTPase domain highlights the importance of this arginine residue in the pathogenesis of the disease.

In addition to LRRK2/dardarin, PINK1, which was previously cloned independently as BRPK, is also a protein kinase. A BLAST homology search revealed that PINK1/BRPK shares sequence homology with the N-terminal half of the *EEED8.9* gene product in *C. elegans*, whereas the C-terminal half of EEED8.9 shares sequence homology with human BRAP2 (BRCA1-interacting protein 2) (Nakajima et al., 2003). The finding that PINK1/BRPK and BRAP2 homologs occur as separate domains in a fusion protein in a lower organism suggests that the mammalian proteins interact functionally and/or physically in a common pathway (Marcotte et al., 1999). BRAP2 has also been named IMP (impedes mitogenic signal propagation) and is a Ras-responsive E3 ubiquitin ligase (Matheny et al., 2004). Upon activation of Ras, IMP is modified by autopolyubiqutination, which releases the inhibition of Raf-MEK complex formation. Thus, PINK1 appears to be involved in Ras/MAPK signaling. A possible link of LRRK2 to Ras/MAPK signaling is suggested by the presence of a Ras-like small GTPase domain in LRRK2, since the MAPK cascade can function as an effector mechanism for the action of small GTPases.

One further point of interest in the PARK8 families is the neuropathological heterogeneity. Affected individuals generally exhibit either typical PD or LBD, in association with neuronal loss and gliosis in the substantia nigra. However, nigral degeneration was associated with a variety of pathologic features: absence of other distinctive pathologic features ("pure nigral degeneration"); brainstem Lewy bodies, typical of PD; widespread brainstem and cortical Lewy bodies, consistent with LBD; or absence of Lewy bodies but presence of neurofibrillary tangles, consistent with tauopathy (Funayama et al., 2002; Paisán-Ruíz et al., 2004; Wszolek et al., 2004; Zimprich et al., 2004). Pure nigral degeneration is also characteristic of *PARK2* cases caused by parkin mutations, whereas parkinsonism with tauopathy is characteristic of some FTDP-17 (frontotemporal dementia with parkinsonism linked to chromosome 17) cases caused by tau mutations. As noted above, LBD can also be caused by α-synuclein mutations. Thus, the clinical, pathologic, and genetic heterogeneity associated with familial parkinsonism suggests that multiple pathogenic mechanisms can lead to the central pathogenic event of nigral degeneration, which produces parkinsonian phenotypes, along with variable other pathological features.

As these observations illustrate, neither Lewy body formation nor NFT formation are obligate events in nigral degeneration, and mutations in a single gene (*LRRK2*) can apparently produce nigral degeneration in the absence or presence of either of these intraneuronal aggregates. Nevertheless, mutations in the α-synuclein and tau genes are sufficient to produce familial parkinsonism with some clinical and neuropathological heterogeneity. A central challenge for research on parkinsonism will be to define these multiple pathogenic mechanisms and their points of molecular convergence. It will be of particular interest to determine whether and how the LRRK2 protein and its putative GTPase and tyrosine kinase activities interact with the other gene products or pathways thus far implicated in familial parkinsonism.

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AMPA Receptors Bring On the Pain

The role of Ca^{2+}-permeable AMPA receptors in pain processing has not been extensively studied. In this issue of *Neuron*, Hartmann et al. show that altering the levels of these receptors has consequences for inflammatory pain hypersensitivity but not acute pain processing.

In the late 1980s and early 1990s, the previously elusive receptors for the excitatory amino acid glutamate were characterized, cloned, and intensively studied. The NMDA receptor was shown to be blocked by Mg^{2+} and to be permeable to Ca^{2+}. The non-NMDA receptors, named the AMPA and kainate receptors, were initially believed to show little permeability to Ca^{2+}. At that time, the kainate receptor's role in nervous system function was obscure, but the AMPA receptors were known as the key transducers of synaptically released glutamate. Their apparent lack of Ca^{2+} permeability and voltage-dependent activity made them seemingly simple to understand compared to the complex NMDA receptor. However, it soon became clear that within the four genes coding subunits of the AMPA receptor family, GluR1, -2, -3, and -4, also called GluR-A, -B, -C, and -D, the presence of GluR2/B made the multisubunit AMPA receptors Ca^{2+} impermeable. In the absence of GluR2/B, AMPA receptors were permeable to Ca^{2+} and showed an inwardly rectifying dependence on membrane potential (Verdoorn et al., 1991). These Ca^{2+}-permeable AMPA receptors are now known to be expressed at synapses between a variety of neurons throughout the central nervous system, including the spinal cord dorsal horn—the first stage of central somatosensory processing.

Ca^{2+}-permeable AMPA receptors have proven notoriously difficult to study. Because they are defined by the absence of GluR2/B, the only certain way to demonstrate their active presence on the surface of a neuron is by functional assay. Pharmacology of the receptors is also limited because the compounds that block them, including polyamines such as Joro spider toxin, are open channel blockers and so are complicated to use. In addition, these compounds are nonselective in that they also block Ca^{2+}-permeable kainate receptors.

In this issue of *Neuron*, Hartmann et al. (2004) have instead used a genetic approach to investigate the role of Ca^{2+}-permeable AMPA receptors in the spinal cord dorsal horn in normal nociceptive processing versus the altered sensory processing present in pathological pain states. The superficial dorsal horn, the main site of termination of nociceptive afferent input to the spinal cord, has previously been shown to contain a high density of Ca^{2+}-permeable AMPA receptors, as identified using kainate-induced cobalt uptake in which cobalt goes through open Ca^{2+}-permeable AMPA receptors (Engelman et al., 1999). Hartmann et al. have shown that, compared with wild-type littermates, mice lacking GluR1/A have reduced numbers of cobalt-positive neurons, whereas mice lacking GluR2/B have increased numbers of cobalt-positive neurons, using this same assay. In other words, mice lacking GluR1/A and GluR1/B have decreased and increased numbers of Ca^{2+}-permeable AMPA receptors in the superficial dorsal horn, respectively.

The AMPA receptor-mediated component of synaptic currents, recorded from the superficial dorsal horn, were reduced in the *GluR1/A*$^{-/-}$ mice and enhanced in the *GluR2/B*$^{-/-}$ mice. However, both groups of mice showed intact nociceptive withdrawal reflexes as assessed behaviorally. Latency to tail flick withdrawal from noxious heat was unaltered, and hindpaw withdrawal in response to noxious thermal or mechanical stimuli was similarly unaffected. Additionally, in an in vitro preparation, activity reflecting spinal cord reflexes elicited by nociceptor stimulation was not altered. This indicates that the nociceptive pathway is intact and functioning in both sets of modified animals.

The behavioral hypersensitivity that develops following an inflammatory insult, however, was altered. The formalin test was used as one assay for such changes. In this test, formalin injection into the hindpaw of the animal results in an early (phase I) and late (phase II) behavioral response. The phase I response reflects C fiber activation due to the inflammatory insult, whereas the phase II response is thought to reflect central sensitization within the dorsal horn, in part because of its sensitivity to intrathecal NMDA receptor antagonists (Woolf and Salter, 2000). *GluR2/B*$^{-/-}$ animals showed an enhanced response in phase II of the formalin test. Conversely, the *GluR1/A*$^{-/-}$ animals showed depressed responses in phase II. This suggests that altering AMPAR composition can modulate NMDAR-mediated increases in central excitability. Consistent with this observation, the GluR1/A animals did not show elevated phosphorylation of extracellular activated MAP Kinases1/2 (ERK1/2) in lamina I following high-frequency stimulation of high-threshold C fibers, an in vitro correlate of the persistent nociceptor activation induced by an inflammatory agent like formalin, whereas *GluR2/B*$^{-/-}$ animals showed normal elevations.

These experiments clearly show that the composition of AMPA receptors expressed at synapses in the pain pathway strongly influences the generation of hypersensitivity in inflammatory pain models. The correlation between altered central sensitization and the numbers of neurons expressing Ca^{2+}-permeable AMPA receptors, as shown by changes in cobalt loading, suggests that this family of receptors can shift the balance of long-lasting changes in excitability within the dorsal horn. To understand the implications of this observation, however, a great deal more investigation will be needed. This is because not only can AMPA receptor type influ-