

Biochemical and molecular features of LRRK2 and its pathophysiological roles in Parkinson's disease

Wongi Seol*

Institute for Brain Science & Technology/Graduate Program of Neuroscience, Inje University, Busan 614-735, Korea

Parkinson's disease (PD) is the second most common neurodegenerative disease, and 5-10% of the PD cases are genetically inherited as familial PD (FPD). LRRK2 (leucine-rich repeat kinase 2) was first reported in 2004 as a gene corresponding to PARK8, an autosomal gene whose dominant mutations cause familial PD. LRRK2 contains both active kinase and GTPase domains as well as protein-protein interaction motifs such as LRR (leucine-rich repeat) and WD40. Most pathogenic LRRK2 mutations are located in either the GTPase or kinase domain, implying important roles for the enzymatic activities in PD pathogenic mechanisms. In comparison to other PD causative genes such as parkin and PINK1, LRRK2 exhibits two important features. One is that LRRK2's mutations (especially the G2019S mutation) were observed in sporadic as well as familial PD patients. Another is that, among the various PD-causing genes, pathological characteristics observed in patients carrying LRRK2 mutations are the most similar to patients with sporadic PD. Because of these two observations, LRRK2 has been intensively investigated for its pathogenic mechanism (s) and as a target gene for PD therapeutics. In this review, the general biochemical and molecular features of LRRK2, the recent results of LRRK2 studies and LRRK2's therapeutic potential as a PD target gene will be discussed. [BMB reports 2010; 43(4): 233-244]

INTRODUCTION

Parkinson's disease

Parkinson's disease (PD; OMIM #168600) was first described by an English physician, James Parkinson, in 1817. PD is a progressive, late-onset disease that occurs in approximately 1% of the population more than 65 years old. It is the second most common neurodegenerative disease next to Alzheimer's disease.

PD's major symptoms are generally bradykinesia, resting

tremor, rigidity, gait difficulty and poor balance, which are related to neuromotor functions, but PD can also sometimes be accompanied by change in mental status, such as dementia and depression (1-3).

Two major pathological hallmarks of PD are specific and progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, located in the midbrain, and formation of Lewy bodies in surviving neurons. Death of the dopaminergic neurons results in loss of motor functions by gradual decrease of dopamine, which is an important neurotransmitter that regulates neuromotor functions. There is currently no cure for PD. At present, L-DOPA (L-dihydroxyphenylalanine), a precursor of dopamine that can pass through the blood-brain barrier (BBB) and is converted to dopamine in the brain, is used as a symptomatic drug for patients with an early stage of PD (4). However, after significant progress of the disease, when the number of surviving dopaminergic neurons is too small to convert L-DOPA to dopamine, L-DOPA loses its effectiveness. At the present time, a surgical method, deep brain stimulation (DBS), is used to effectively lessen symptoms of some PD patients in this stage (5).

The main cause or exact mechanism of PD pathogenesis is yet unknown. However, increase of misfolded protein, mitochondrial dysfunction and cellular oxidative stress are generally considered as major causes of sporadic PD generation.

Genetics of Parkinson's disease

Although most PD cases occur sporadically, 5-10% of the cases are genetically inherited as familial PD (FPD). Through analysis of FPD patients' samples during the last two decades, more than ten PARK loci have been mapped, and most of genes corresponding to the loci were identified (3, 6, 7; Table 1). Most FPD genes can be inherited as either autosomal dominant or autosomal recessive forms. Examples of the recessive genes are *Parkin*, *PINK1* and *DJ-1*, the first two of which are related to juvenile PD. The representative genes for the autosomal dominant FPD are *SNCA* (α -synuclein) and *LRRK2* (Leucine-rich repeat kinase 2). α -Synuclein, a major component of Lewy bodies, was identified as a protein coded by a gene corresponding to both PARK1 and PARK4. PARK1 and PARK4 were originally reported as a mutated form of α -synuclein (8) and a duplication or triplication of the wild type α -synuclein gene (9-11), respectively. These genetic studies of

*Corresponding author. Tel: 82-51-892-4184; Fax: 82-51-892-0059; E-mail: wseol@inje.ac.kr

Received 31 March 2010

Keywords: GTPase, G2019S, Kinase, LRRK2, Parkinson's disease

Table 1. PD genes identified in FPD cases

Park locus	Chromosome location	Gene	Functions	Mode of inheritance	Reference
PARK1, 4	4q21	<i>Alpha-synuclein (SNCA)</i>	Component of Lewy body	AD	9, 10, 109
PARK2	6q25-27	<i>Parkin</i>	E3 Ubiquitin protein ligase	AR	27
PARK3	2p13	Unknown	Unknown	AD	110
PARK5	4p14	<i>UCHL1*</i>	Ubiquitin C-terminal hydrolase	AD	111
PARK6	1p35-36	<i>PINK1</i>	Mitochondrial kinase	AR	112
PARK7	1p36	<i>DJ1</i>	Antioxidation	AR	113
PARK8	12q12	<i>LRRK2</i>	Kinase, GTPase	AD	12,13
PARK9	1p36	<i>ATP13A2</i>	Probable cation-transporting ATPase	AR	114
PARK10	1p32	Unknown	Unknown	AD	115
PARK11	2q36-37	<i>GIGYF2*</i>	Regulation of tyrosine kinase receptor signaling?	AD	116
PARK12	Xq21-25	Unknown	Unknown	X chromosome	117
PARK13	2p13	<i>HTRA2</i>	Serine protease	Unknown	118
PARK14	22q13.1	<i>PLA2G6</i>	Phospholipases A2	AR	119
PARK15	22q12-13	<i>FBXO7</i>	E3 ubiquitin protein ligase	AR	120, 121
PARK16	1q32	Unknown	Unknown	Unknown	14

*There are conflicting reports on whether *UCHL1* and *GIGYF2* are truly the corresponding PARK genes (122-124).

α -synuclein indicated that not only expression of pathogenic mutants, but also over-expression of wild type α -synuclein can cause PD. LRRK2 was first reported in 2004 as a gene corresponding to PARK8 (12, 13). Since then, LRRK2 has been intensively investigated because its mutations are observed in FPD as well as sporadic PD patients, and pathological characteristics of PD patients with the LRRK2 mutations are similar to patients with sporadic PD. The details of LRRK2 biology and pathology will be discussed in the following sections. PARK16 is the most recently reported PARK locus, discovered via a genome-wide association study for PD (14).

LRRK2

Funayama *et al.* reported PARK8 as a new PARK locus from a genome-wide linkage study of a Japanese family that exhibited autosomal dominant Parkinson's disease (15). In 2004, two groups concurrently reported identification of a gene corresponding to PARK8 (12, 13). The protein encoded by PARK8 was named as either LRRK2 (Leucine-rich repeat kinase 2; 12) or dardarin (13).

LRRK2 (dardarin) is a large protein, consisting of 2527 amino acids, whose molecular weight is approximately 285 kD. It is a multi-domain protein containing distinctive enzymatic domains of a GTPase and a kinase, along with the protein interaction motifs LRR (Leucine-rich repeat) and WD40 (Fig. 1A). The domain structure of LRRK2 indicates that LRRK2 belongs to the ROCO family, whose members contain both small GTPase (ROC) and kinase domains as well as the COR (C-terminal of Roc) region (Fig. 1B; 16). Most ROCO family members also contain one or both of the LRR and WD40 domains (16). LRRK1 is a homologue of LRRK2, and is also a mammalian member of the ROCO family (17). Both the GTPase and kinase domains of LRRK2 and LRRK1 were shown to be func-

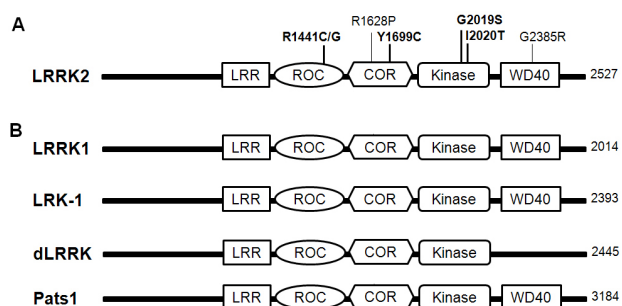


Fig. 1. (A) Schematic representation of LRRK2 and its domains with PD-specific pathogenic mutations (bold letters with thick lines). R1628P and G2385R, two mutations usually found in Asian populations and thought to be risk factors for PD, are also indicated. (B) The domain structures of ROCO family members. LRR: Leucine-rich repeat, ROC: Ras in complex, COR: C-terminal of Roc. The NCBI accession numbers of each protein are: LRRK2 (human), NP_940980; LRRK1 (human), NP_078928; LRK-1 (worm), NP_492839; dLRRK (fly), NP_001097847; and Pats1 (*Dictyostelium discoideum*), XP_645923. Most papers suggested the presence of ankyrin repeats, another protein interaction domain, in the N-terminus of the LRR domain (50, 104). The scale of each domain or protein is not proportional to its actual size.

tionally active (17-19). The kinase domain of LRRK2 was reported to share homology with mixed-lineage kinase (MLK; 18) and receptor interacting protein kinase (RIPK; 20). Although both LRRK1 and LRRK2 are present in mammals such as human, rat and mouse, only one orthologue of LRRK was identified in *Drosophila* and *C. elegans*, respectively called dLRRK and LRK-1 (21, 22).

LRRK2 spans an approximately 144 kb region of chromosome 12, contains 51 exons and is ubiquitously expressed, al-

though its expression is more abundant in the kidney and lung (12, 13). Due to conflicting data, there was some debate on whether LRRK2 is expressed in the substantia nigra pars compacta, the region in which degeneration of dopaminergic neurons first starts in PD patients (23, 24). However, data reported later indicated that LRRK2 is indeed expressed in dopaminergic neurons in the substantia nigra, although its expression is relatively low (25, 26). Some of the PARK genes (parkin and PINK1) are also expressed ubiquitously, or at least in several tissues in addition to the brain (27, 28). The non-specific and relatively ubiquitous expression pattern of these genes suggested that death of midbrain dopaminergic (DA) neurons might be due to failure in the cellular functions of DA neurons mediated by the mutated proteins, and not due to expression itself of PARK genes.

Investigation of LRRK2's subcellular location revealed that most LRRK2 is present in the cytosol, and a portion of them is associated with the membranes of organelles such as the mitochondria, golgi apparatus, and synaptic vesicles. LRRK2 is also concentrated in lipid rafts (18, 29, 30).

Pathogenic LRRK2 Mutations

The first two reports of LRRK2 identified several PD-specific mutations, I1122V, R1441G/C, Y1699C and I2020T, in addition to a point mutation (3342A>G) near the exon/intron border (12, 13). G2019S, another pathogenic mutation in LRRK2, was simultaneously reported by three groups shortly after (31-33). In addition, G2385R and R1628P mutations were found in the Asian PD population (34, 35). A case-controlled study with a large sample size revealed that G2019S is the most prevalent mutation, present in more than 85% of PD patients carrying LRRK2 mutations, with R1441C/G as the next most prevalent, at approximately 10% (36). In addition, the G2019S mutation was observed in 4% of all familial PD cases. Strikingly, the G2019S mutation was also identified in 1% of sporadic PD cases, suggesting that the mutation plays a critical role in the etiology of PD (36). In certain ethnic populations, the frequency of the G2019S mutation was significantly higher than that in others. For example, 13-40% of all PD patients in Ashkenazi Jewish and northern African Arab populations have the G2019S mutation (37-40). In Asian populations, the presence of G2019S in PD patients is extremely low, with a frequency less than 0.1% (41), but G2385R and R1628P are more frequently present, suggesting both mutations may be PD risk factors (34, 42). Besides these PD-specific mutations, there are several polymorphic base changes in the LRRK2 gene (43). However, although mutations of both LRRK2 and α -synuclein are inherited in an autosomal dominant manner, duplication of the wild type LRRK2 gene has not yet been observed while duplication and triplication of the wild type α -synuclein has been found in the PARK4 locus (9-11).

The penetrance of the G2019S mutation increases with age and reaches more than 74 % in 70 year olds (36, 40). However, an octogenarian with the mutation was reported to survive

without development of PD symptoms (44). In contrast, a recent study suggested that specific genetic backgrounds, such as the polymorphic variations in *MAPT* (tau gene), change the age of disease onset in individuals carrying the pathogenic LRRK2 mutations (45).

PD patients carrying the pathogenic LRRK2 R1441C or G2019S mutation exhibited clinical symptoms similar to idiopathic PD (31, 46). Most of the patients showed late disease onset and symptoms of rigidity, bradykinesia, tremor, postural instability and levodopa response, which are similar to the sporadic PD cases (31). This similarity highlights LRRK2 as a potential PD therapeutic target gene among the familial PD genes.

Molecular Biological Features of LRRK2 and Its Pathological Roles in Parkinson's Disease

Dual enzymatic activities of LRRK2

Since its discovery, LRRK2 was highly noticed because of its dual enzyme activity domains in addition to its relation to PD. So far, LRRK2 and LRRK1 are the only two enzymes that contain both GTPase and kinase activities, which play critical roles for signal transduction. Therefore, it became important to determine whether the PD-specific mutations change the enzymatic activities of these proteins.

Kinase: Protein phosphorylation is one of the most important tools to regulate cellular functions. Since the kinase domain of LRRK2 is similar to MAPKKK, an upstream kinase of cellular signal transduction, and because both the G2019S and I2020T mutations are located in the conserved kinase domain, the effect of the LRRK2 mutants on its kinase activities was heavily investigated. Several groups reported kinase activities of the mutants using *in vitro* kinase assays and found that only the G2019S protein exhibited increased kinase activity, while the other mutants, R1441C, Y1699C and I2020T, showed ambiguous and sometimes conflicting results (18, 19, 47-49). These conflicting data may be due to the use of different substrates in the kinase assays, namely the use of general kinase substrates, such as myelin basic protein or synthetic peptide, or may be due to different enzyme preparations, which were expressed from bacterial, insect, or mammalian cells and used a variety of different tags at either the N- or C-terminal ends (50, 51). The increased kinase activity of the G2019S mutant could explain the dominant heredity of that PARK8 mutation. Therefore, isolation of specific kinase inhibitor(s) of LRRK2 could lead to developing new PD therapeutics.

In addition to the value of the LRRK2 kinase as a therapeutic target, identification of the physiological substrates of LRRK2 may reveal the pathogenic mechanism of LRRK2 in PD. Several reports have raised various proteins as possible substrates for the LRRK2 kinase. The first, moesin, has a potential phosphorylation site at Thr558 (47). Moesin is a member of the ERM (ezrin, radixin, and moesin) family, which functions

as an anchor for the cytoskeleton to cytoplasmic membranes (52). Parisiadou *et al.* later showed that cultured neurons derived from G2019S transgenic mice had significantly higher numbers of phosphorylated ERM-positive and F-actin-enriched filopodia (53). This increase resulted in retardation of neurite outgrowth, a representative phenotype repeatedly observed in cells over-expressing G2019S (53). Another potential LRRK2 substrate is β -tubulin, which, in studies that purified the protein from bovine brains, has been reported to be specifically phosphorylated by LRRK2 at Thr107, again suggesting a critical role of LRRK2 in regulation of cytoskeleton stability (54).

4E-BP, a eukaryotic initiation factor 4E (eIF4E)-binding protein (BP), has been also reported as a LRRK2 kinase substrate (55). Phosphorylation of 4E-BP by LRRK2 prevents its inhibitory binding to eIF4E and, thus stimulates eIF4E-mediated protein translation and increases oxidative stress-induced neurotoxicity in DA neurons in *Drosophila* (55). This observation was supported by a discovery that loss of dLRRK activated 4E-BP, resulting in suppression of *Drosophila* parkin and PINK1 pathogenic phenotypes (56). The stimulation of protein translation by LRRK2-mediated 4E-BP phosphorylation might explain why co-transfection of LRRK2 with cDNA genes enhanced synthesis of the corresponding protein when compared to transfection of the cDNA alone, generally regardless of the gene identity (unpublished result, Seol, W *et al.*). However, a recent report found that 4E-BP phosphorylation by LRRK2 is weaker than LRRK2 autophosphorylation *in vitro*, raising some questions as to whether 4E-BP is a physiological substrate of LRRK2 (57). Members of the MAPKK family, MKK3, 4, 6 and 7, were also identified as substrates for LRRK2 kinase, and PD-specific LRRK2 mutants enhanced binding to and phosphorylation of these MKKs (58, 59). These observations suggest that LRRK2 indeed functions as a MAPKKK.

LRRK2's kinase activity was predicted to be a Ser/Thr kinase. An analysis of autophosphorylated LRRK2 by matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry revealed that Ser1403, Thr1404, Thr1410, Thr1491 in the ROC domain, as well as Thr1967 and Thr1969 in the kinase domain, can all be autophosphorylated (60). Kinase assays of LRRK2 using various synthetic peptides showed that the enzyme prefers to phosphorylate Thr over Ser residues, and one study developed a synthetic peptide, Nictide, as a LRRK2 substrate for *in vitro* kinase assays (61).

GTPase: The GTPase domain of LRRK2 shows sequence homology to the small GTPase family. The small GTPase family functions as a molecular switch that requires GEF (guanine nucleotide-exchange factor) and GAP (GTPase-activating protein) activity to shuttle between its GTP-bound active state and GDP-bound inactive state (62). Therefore, proving whether the GTPase domain of LRRK2 is functional was difficult at first without knowing the identity of LRRK2's specific GEF. However, several groups successfully showed activity by the GTPase domain of LRRK2, though its function was weak (63-66). These

groups showed that LRRK2 can bind to GTP, and that the PD-specific R1441C mutation, located in the GTPase domain, does not increase GTP binding ability, but decreases GTP hydrolysis activity, increasing the duration of the enzyme's active status (63-65). It is not yet clear if functional GTPase activity is required for kinase activity. Several groups showed that the dominant negative mutants of the GTPase domain (K1347A or T1348N), which are unable to bind to GTP, significantly weaken LRRK2 kinase activity (19, 48, 67) whereas others reported that loss of LRRK2 binding to GTP, GDP or GMP had no effect on its kinase activity (68). Similarly, studies for the kinase activity of the R1441C mutant have found conflicting results, with decreasing (63, 64) or no change (47, 69) of the activity.

So far, LRRK2's GTPase activity has been assayed without any known, exogenous GEF protein. This observation suggests the possibility that LRRK2 belongs to a novel subgroup of GTPases that, instead of requiring a GEF protein, utilizes homodimerization of the GTPase domain for its GTPase activity, like dynamin (70). In fact, the crystal structure of the LRRK2 ROC domain complexed with GDP revealed a dimeric fold with the R1441 site located at the interface of two monomers (66). Interestingly, LRRK2 homodimerization is also required for its kinase activity (71, 72), implying cooperation between the two enzyme activities.

In conclusion, the accumulated data suggest that LRRK2 contains functional GTPase and kinase activities. Of the PD-specific mutants, G2019S enhances kinase activity and R1441C decreases GTPase activity. However, it remains to be clarified whether any mutants other than G2019S affect its kinase activity and whether GTPase activity is a prerequisite for kinase activity. Further studies are necessary to resolve potentially conflicting data on this subject.

Proteins interacting with LRRK2

To understand the physiological functions of novel proteins like LRRK2, one approach is to identify well-characterized proteins that interact with the protein of interest and to use the known physiological roles of those proteins to deduce the cellular functions of the novel protein. Since the identification of LRRK2, efforts to isolate proteins that interact with it have used a variety of methods, such as yeast two-hybrid screening (30), protein pull down (73) and co-immunoprecipitation assays (74). Dachsel *et al.* used a method combining protein immunoprecipitation with tandem mass spectrometry and identified three major groups of LRRK2-interacting proteins: (i) proteins related to the chaperone-mediated response, (ii) proteins functioning in the cytoskeleton and trafficking and (iii) proteins with kinase activity (74). Subsequent reports confirmed that most LRRK2-interacting proteins belong to these groups (73, 75, 76), although the interacting proteins identified in these reports differed from those isolated by Dachsel *et al.* (74). Gandhi *et al.* showed that the ROC domain of LRRK2 can interact with α/β -tubulin in a GTP-independent manner, and this interaction was not affected by the R1441C mutation (73). Later, β -tubulin

was reported to be a LRRK2 kinase substrate (54). Heat shock protein 90 (Hsp90) is another protein that complexes with LRRK2. Treatment with Hsp90 inhibitor prevents the association of LRRK2 with Hsp90 and results in proteasomal degradation of LRRK2 (75). Another study reported that the carboxyl terminus of Hsp70-interacting protein (CHIP) promotes LRRK2 degradation in a proteasome-dependent manner by ubiquitination of LRRK2 (76). Consequently, over-expression of CHIP alleviates toxicity of the LRRK2 mutants (76). Moreover, Ding *et al.* showed that Hsp90 can attenuate CHIP-mediated LRRK2 degradation (77). These results together revealed an important mechanism to regulate LRRK2 stability. The I2020T PD-specific mutant was also reported to be less stable than the wild type (78, 79).

Another report found that LRRK2 interacts with Rab5b, a key regulator of early endocytosis (30). Interestingly, over-expression of the wild type LRRK2 decreased the rate of endocytosis. Co-expression of active, but not inactive, Rab5b rescues this endocytosis deficit in primary neuron cultures (30). It was also shown that FADD (Fas-associated protein with death domain), a death adaptor protein critical for apoptosis activation, interacts with LRRK2, and inhibiting FADD function prevented LRRK2-mediated neuronal cell death in primary neuronal cultures (80). This result provided a direct link between neuronal degeneration caused by LRRK2 and activation of apoptosis. It was also reported that LRRK2 interacts with the dishevelled family (DVL1-3), a key regulator of Wnt signaling pathways, suggesting that LRRK2 might be involved in signaling pathways that are important for axon guidance, synapse formation and neuronal maintenance (81).

From the beginning of LRRK2 research, it has been of great interest whether LRRK2 interacts with other FPD proteins and affects their functions. Several reports suggested that LRRK2 interacts with Parkin, DJ-1 or PINK-1 in mammalian cell lines, *C. elegans*, and *Drosophila melanogaster* models of Parkinson's disease (82-84). Another recent study revealed that over-expression of LRRK2 accelerates the progression of neuropathological phenotypes in transgenic mice with the A53T α -synuclein mutation (85). In addition, Parkin alleviated dopaminergic neuronal loss mediated by over-expression of LRRK2 G2019S mutants (86). Combined together, these reports suggest functional linkages among the various FPD genes.

Identification of more LRRK2-interacting proteins, along with their cellular and physiological functions and their effects on LRRK2, will further extend understanding of LRRK2's pathophysiological roles in PD.

Normal and pathological features of LRRK2

Functions of wild type LRRK2 and its pathogenic mutants were intensively studied under both normal physiological and abnormal stress conditions to discover the mechanisms of etiology of LRRK2-mediated PD. Over-expression of wild type LRRK2 induces neuronal cell death, neurite shortening, protein aggregation, oxidative stress-induced cell death and increased

levels of intracellular reactive oxygen species (ROS). Over-expression of the pathogenic mutants, especially G2019S, aggravates most of these phenomena (19, 48, 49, 69, 82, 87-89). Biochemical and molecular approaches revealed that these phenotypes were mostly abolished by expression of kinase-dead mutants of LRRK2, K1906A/M, D1994N/A or DY2017-2018AL, suggesting that most toxic effects of LRRK2 over-expression are caused by its kinase activity (19, 48, 49, 87). However, the residual kinase activities of these kinase-dead mutants are variable in *in vitro* kinase assays, requiring caution when studying and interpreting data on these mutants.

LRRK2 over-expression reduced the rate of endocytosis, but expression of the pathogenic mutants G2019S or R1441C, or of the kinase-dead K1906M mutant, did affect endocytosis in comparison with the wild type protein (30), suggesting that the endocytosis-related phenotype is not mediated by LRRK2 kinase activity.

Both autophagy (87, 90) and apoptosis (49, 58, 91) have been suggested as mechanisms for LRRK2-dependent dopaminergic neuronal cell loss. Based on the interaction of LRRK2 with MKKs and the sequence homology of LRRK2 to MAPKKK, LRRK2 could be a signal transducing kinase in the MAPK pathway. In fact, treatment with an extracellular signal-regulated kinase (ERK) pathway inhibitor alleviated G2019S LRRK2-induced neuronal cell death and neurite shortening (87-89). Moreover, over-expression of LRRK2 activates ERK, resulting in slight induction of α -synuclein expression, which could be suppressed by treatment of a specific ERK inhibitor (92).

A useful approach to elucidate gene function has been the development of transgenic, knock-out or knock-in animals and comparison of their physiological characteristics with that of their wild type littermates. In addition, for disease-related genes, if these animals showed phenotypes similar to those of patients with the disease, they can be utilized as model organisms for therapeutic development. Because the pathogenic LRRK2 mutations are dominantly inherited, transgenic LRRK2 phenotypes, especially that of pathogenic G2019S, are of great interest. Presently, there is no report of any transgenic animals generated by insertion of cDNA of wild type or mutated LRRK2 that exhibit any similarity to PD pathology. The LRRK2 transgenic animals generated by insertion of a BAC containing the R1441G mutation recapitulated most PD pathological symptoms, including age-dependent tremors associated with diminished dopamine release and levodopa-responsiveness (93). Another study showed that R1441C knock-in animals appeared normal up to 2 years of age. However, careful analysis revealed decreased amphetamine (AMPH)-induced locomotor activity, suggesting impairment in dopamine neurotransmission (94). A recent report on two kinds of BAC transgenic mice showed that expression of wild type LRRK2 enhanced striatal dopamine transmission and motor performance, whereas expression of G2019S mutants decreased striatal DA content as well as its release and uptake in an age-dependent manner (95).

Efforts to generate LRRK2 transgenic or deletion mutant animals were also attempted in flies and worms, two very useful experimental animals for this purpose. Abnormal localization of SNB-1, an orthologue of mammalian synaptobrevin2/VAMP2, in both presynaptic and dendritic ends has been reported in the deletion mutant of LRRK-1, an orthologue of LRRK2 in *C. elegans* (22). This result suggested that LRRK2 may be involved in regulation of specific vesicle/cargo transport to proper neurites. Expression of human LRRK2 WT and G2019S in *C. elegans* increased survival after treatment with paraquat or rotenone, agents that induce mitochondrial dysfunction, implying that LRRK2 functions in mitochondria (96).

Drosophila LRRK2 models were also developed (21, 97, 98). Loss-of-function dLRRK mutants in *Drosophila* yielded conflicting results on locomotive motor impairment (21, 97). However, a gain-of-function model was reported to mimic various features of LRRK2-mediated PD pathology, implying that this type of fly could be utilized as a useful model for development of PD therapeutics (98).

Two experiments are particularly interesting because they used leukocytes from PD patients with the G2019S mutation (99, 100). One study performed protein array analysis to compare MAPK activity between patients and healthy controls and reported that phosphorylation of Src, c-JNK and heat shock protein 27 (HSP27) in patients' samples are lower than in the controls (99). Mutez *et al.* carried out a microarray experiment using peripheral blood mononuclear cells of PD patients with the G2019S mutation. Their analysis detected differentially expressed genes common to the PD patients in comparison with the healthy controls, and ontology analysis of the genes revealed perturbation of pathways related to PD-related neurodegeneration, such as ubiquitin-regulated protein degradation, mitochondrial oxidation, axonal guidance, inflammation and apoptosis (100). Metabolomic analysis was also applied to plasma samples from PD patients with or without the LRRK2 G2019S mutation, along with healthy controls (101). The results showed that the metabolome pattern was clearly segregated among different groups and that uric acid levels were significantly reduced both in sporadic and LRRK2-carrying PD patients (101), which was previously reported using post-mortem substantia nigra tissue samples from both PD patients and controls (102). These results raise the promising possibility that, to diagnose PD and to measure effectiveness of treatment, patient blood can be utilized. Further research using the patients' blood samples should be performed to develop a suitable surrogate marker of PD, a critical issue in PD diagnosis.

LRRK2 as a PD Therapeutic Target Gene

As previously mentioned, LRRK2 mutations are highly prevalent in both familial and sporadic PD cases, and, among various FPD genes, phenotypes of patients with LRRK2 mutations are the most similar to the sporadic PD patients' phenotypes. As a result, LRRK2 has been thought to be a promising target

gene for PD therapeutic development. Originally, there was significant doubt over whether specific kinase inhibitors could be developed, since most kinases are considerably conserved among their functional ATP- or Mg²⁺-binding domains. However, continuous efforts generated kinase inhibitors with sufficient selectivity and, currently, several small molecule kinase inhibitors are on the market. For example, Imatinib (Novartis' Gleevec) is used to treat chronic myelogenous leukemia caused by the bcr-abl type of mutated tyrosine kinase. Others are Gefitinib (AstraZeneca's Iressa) and Erlotinib (OSI/Roche's Tarceva), drugs that target the kinase function of EGFR (epidermal growth factor receptor) and are being used to treat non-small cell lung cancer and pancreatic cancer. Because LRRK2 mutations are inherited in a dominant manner and the most prevalent mutation, G2019S, increases kinase activity, it will be easier to develop a LRRK2 kinase inhibitor instead of developing a drug to enhance function of the FPD recessive genes. Two recent papers tested LRRK2 kinase activity with several known kinase inhibitors (61, 103). Nichols *et al.* tested the effectiveness of three Rho kinase (ROCK) inhibitors and showed that Y-27632 and H-1152, but not GSK429286A, suppressed LRRK2 with similar potency as their activity against ROCK (61). Covy *et al.* used well-known kinase peptide substrates to demonstrate that LRRK2 contains remarkable substrate specificity and that small molecules with a basic indolocarbazole structure potentially inhibit LRRK2 kinase activity (103). Further efforts to isolate small molecules that selectively inhibit LRRK2 kinase activity may result in development of a novel type of PD drug.

CONCLUSION

Since the identification of LRRK2 as the product of the PARK8 gene six years ago, extensive research has been focused on elucidating LRRK2 function in PD pathology as well as in normal physiology. These results have provided clues to better understand PD pathophysiology. However, there still remain several critical issues:

- What are the stimuli to activate LRRK2? Which signal transduction pathways are involved both upstream and downstream of LRRK2?
- What are the differences in kinase and GTPase activities among the various LRRK2 pathogenic mutants? How are these differences relevant to their pathogenicity?
- Is LRRK2 kinase activity modulated by the GTPase domain?
- Which proteins interact with the LRR and WD40 domains of LRRK2, and do any of them regulate LRRK2 function?
- Why do some carriers of G2019S remain asymptomatic? Is it due to differences in genetic background? If so, what are the genes responsible?

While a cure for Parkinson's disease may not be available in the near future, the combined efforts of academia, industry and non-profit organizations like the MJFF (The Michael J. Fox

Foundation) to study LRRK2 will allow a better understanding of and the development of further treatments for PD.

This review is intended to provide a general summary of recent findings in LRRK2 research in a limited space. Recently, a series of excellent reviews on LRRK2 was published in FEBS J. (104-108). I suggest referring to these reviews for more detailed information.

Acknowledgements

I would like to apologize to those colleagues whose important results were not included in this review due to space limitations. Research in my laboratory is supported by an IRGB funded by Inje University in Korea, the Basic Science Research Program (2009-0083924) and the Global Partnership Program (2009-00505) through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science and Technology.

REFERENCES

1. Olanow, C. W. and Tatton, W. G. (1999) Etiology and pathogenesis of Parkinson's disease. *Annu. Rev. Neurosci.* **22**, 123-144.
2. Moore, D. J., West, A. B., Dawson, V. L. and Dawson, T. M. (2005) Molecular pathophysiology of Parkinson's disease. *Annu. Rev. Neurosci.* **28**, 57-87.
3. Thomas, B. and Beal, M. F. (2007) Parkinson's disease. *Hum. Mol. Genet.* **16 Spec No. 2**, R183-R194.
4. Fahn, S. (2006) Levodopa in the treatment of Parkinson's disease. *J. Neural. Transm. Suppl.* **71**, 1-15.
5. Halpern, C., Hurtig, H., Jaggi, J., Grossman, M., Won, M. and Baltuch, G. (2007) Deep brain stimulation in neurologic disorders. *Parkinsonism Relat. Disord.* **13**, 1-16.
6. Lewthwaite, A. J. and Nicholl, D. J. (2005) Genetics of parkinsonism. *Curr. Neurol. Neurosci. Rep.* **5**, 397-404.
7. Belin, A. C. and Westlund, M. (2008) Parkinson's disease: a genetic perspective. *FEBS J.* **275**, 1377-1383.
8. Farrer, M., Wavrant-De Vrieze, F., Crook, R., Boles, L., Perez-Tur, J., Hardy, J., Johnson, W. G., Steele, J., Maraganore, D., Gwinn, K. and Lynch, T. (1998) Low frequency of alpha-synuclein mutations in familial Parkinson's disease. *Ann. Neurol.* **43**, 394-397.
9. Ibanez, P., Bonnet, A. M., Debarges, B., Lohmann, E., Tison, F., Pollak, P., Agid, Y., Durr, A. and Brice, A. (2004) Causal relation between alpha-synuclein gene duplication and familial Parkinson's disease. *Lancet* **364**, 1169-1171.
10. Chartier-Harlin, M. C., Kachergus, J., Roumier, C., Mouroux, V., Douay, X., Lincoln, S., Levecque, C., Larvor, L., Andrieux, J., Hulihan, M., Waucquier, N., Defebvre, L., Amouyel, P., Farrer, M. and Destee, A. (2004) Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet* **364**, 1167-1169.
11. Farrer, M., Kachergus, J., Forno, L., Lincoln, S., Wang, D. S., Hulihan, M., Maraganore, D., Gwinn-Hardy, K., Wszolek, Z., Dickson, D. and Langston, J. W. (2004) Comparison of kindreds with parkinsonism and alpha-synuclein genomic multiplications. *Ann. Neurol.* **55**, 174-179.
12. Zimprich, A., Biskup, S., Leitner, P., Lichtner, P., Farrer, M., Lincoln, S., Kachergus, J., Hulihan, M., Uitti, R. J., Calne, D. B., Stoessl, A. J., Pfeiffer, R. F., Patenge, N., Carbajal, I. C., Vieregge, P., Asmus, F., Muller-Mylhok, B., Dickson, D. W., Meitinger, T., Strom, T. M., Wszolek, Z. K. and Gasser, T. (2004) Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* **44**, 601-607.
13. Paisan-Ruiz, C., Jain, S., Evans, E. W., Gilks, W. P., Simon, J., van der Brug, M., Lopez de Munain, A., Aparicio, S., Gil, A. M., Khan, N., Johnson, J., Martinez, J. R., Nicholl, D., Carrera, I. M., Pena, A. S., de Silva, R., Lees, A., Marti-Masso, J. F., Perez-Tur, J., Wood, N. W. and Singleton, A. B. (2004) Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron* **44**, 595-600.
14. Satake, W., Nakabayashi, Y., Mizuta, I., Hirota, Y., Ito, C., Kubo, M., Kawaguchi, T., Tsunoda, T., Watanabe, M., Takeda, A., Tomiyama, H., Nakashima, K., Hasegawa, K., Obata, F., Yoshikawa, T., Kawakami, H., Sakoda, S., Yamamoto, M., Hattori, N., Murata, M., Nakamura, Y. and Toda, T. (2009) Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat. Genet.* **41**, 1303-1307.
15. Funayama, M., Hasegawa, K., Kowa, H., Saito, M., Tsuji, S. and Obata, F. (2002) A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2-q13.1. *Ann. Neurol.* **51**, 296-301.
16. Bosgraaf, L. and Van Haastert, P. J. (2003) Roc, a Ras/GTPase domain in complex proteins. *Biochim. Biophys. Acta.* **1643**, 5-10.
17. Korr, D., Toschi, L., Donner, P., Pohlenz, H. D., Kreft, B. and Weiss, B. (2006) LRRK1 protein kinase activity is stimulated upon binding of GTP to its Roc domain. *Cell Signal* **18**, 910-920.
18. West, A. B., Moore, D. J., Biskup, S., Bugayenko, A., Smith, W. W., Ross, C. A., Dawson, V. L. and Dawson, T. M. (2005) Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 16842-16847.
19. Smith, W. W., Pei, Z., Jiang, H., Dawson, V. L., Dawson, T. M. and Ross, C. A. (2006) Kinase activity of mutant LRRK2 mediates neuronal toxicity. *Nat. Neurosci.* **9**, 1231-1233.
20. Gandhi, P. N., Chen, S. G. and Wilson-Delfosse, A. L. (2009) Leucine-rich repeat kinase 2 (LRRK2): a key player in the pathogenesis of Parkinson's disease. *J. Neurosci. Res.* **87**, 1283-1295.
21. Lee, S. B., Kim, W., Lee, S. and Chung, J. (2007) Loss of LRRK2/PARK8 induces degeneration of dopaminergic neurons in *Drosophila*. *Biochem. Biophys. Res. Commun.* **358**, 534-539.
22. Sakaguchi-Nakashima, A., Meir, J. Y., Jin, Y., Matsumoto, K. and Hisamoto, N. (2007) LRRK-1, a *C. elegans* PARK8-related kinase, regulates axonal-dendritic polarity of SV proteins. *Curr. Biol.* **17**, 592-598.

23. Simon-Sanchez, J., Herranz-Perez, V., Olucha-Bordonau, F. and Perez-Tur, J. (2006) LRRK2 is expressed in areas affected by Parkinson's disease in the adult mouse brain. *Eur. J. Neurosci.* **23**, 659-666.
24. Melrose, H., Lincoln, S., Tyndall, G., Dickson, D. and Farrer, M. (2006) Anatomical localization of leucine-rich repeat kinase 2 in mouse brain. *Neuroscience* **139**, 791-794.
25. Higashi, S., Moore, D. J., Colebrooke, R. E., Biskup, S., Dawson, V. L., Arai, H., Dawson, T. M. and Emson, P. C. (2007) Expression and localization of Parkinson's disease-associated leucine-rich repeat kinase 2 in the mouse brain. *J. Neurochem.* **100**, 363-381.
26. Han, B. S., Iacovitti, L., Katano, T., Hattori, N., Seol, W. and Kim, K. S. (2008) Expression of the LRRK2 gene in the midbrain dopaminergic neurons of the substantia nigra. *Neurosci. Lett.* **442**, 190-194.
27. Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y. and Shimizu, N. (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* **392**, 605-608.
28. Unoki, M. and Nakamura, Y. (2001) Growth-suppressive effects of BPOZ and EGR2, two genes involved in the PTEN signaling pathway. *Oncogene* **20**, 4457-4465.
29. Hatano, T., Kubo, S., Imai, S., Maeda, M., Ishikawa, K., Mizuno, Y. and Hattori, N. (2007) Leucine-rich repeat kinase 2 associates with lipid rafts. *Hum. Mol. Genet.* **16**, 678-690.
30. Shin, N., Jeong, H., Kwon, J., Heo, H. Y., Kwon, J. J., Yun, H. J., Kim, C. H., Han, B. S., Tong, Y., Shen, J., Hatano, T., Hattori, N., Kim, K. S., Chang, S. and Seol, W. (2008) LRRK2 regulates synaptic vesicle endocytosis. *Exp. Cell Res.* **314**, 2055-2065.
31. Nichols, W. C., Pankratz, N., Hernandez, D., Paisan-Ruiz, C., Jain, S., Halter, C. A., Michaels, V. E., Reed, T., Rudolph, A., Shults, C. W., Singleton, A. and Foroud, T. (2005) Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease. *Lancet* **365**, 410-412.
32. Di Fonzo, A., Rohe, C. F., Ferreira, J., Chien, H. F., Vacca, L., Stocchi, F., Guedes, L., Fabrizio, E., Manfredi, M., Vanacore, N., Goldwurm, S., Breedveld, G., Sampaio, C., Meco, G., Barbosa, E., Oostra, B. A. and Bonifati, V. (2005) A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease. *Lancet* **365**, 412-415.
33. Gilks, W. P., Abou-Sleiman, P. M., Gandhi, S., Jain, S., Singleton, A., Lees, A. J., Shaw, K., Bhatia, K. P., Bonifati, V., Quinn, N. P., Lynch, J., Healy, D. G., Holton, J. L., Revesz, T. and Wood, N. W. (2005) A common LRRK2 mutation in idiopathic Parkinson's disease. *Lancet* **365**, 415-416.
34. Tan, E. K., Zhao, Y., Skipper, L., Tan, M. G., Di Fonzo, A., Sun, L., Fook-Chong, S., Tang, S., Chua, E., Yuen, Y., Tan, L., Pavanni, R., Wong, M. C., Kolatkar, P., Lu, C. S., Bonifati, V. and Liu, J. J. (2007) The LRRK2 Gly2385Arg variant is associated with Parkinson's disease: genetic and functional evidence. *Hum. Genet.* **120**, 857-863.
35. Ross, O. A., Wu, Y. R., Lee, M. C., Funayama, M., Chen, M. L., Soto, A. I., Mata, I. F., Lee-Chen, G. J., Chen, C. M., Tang, M., Zhao, Y., Hattori, N., Farrer, M. J., Tan, E. K. and Wu, R. M. (2008) Analysis of Lrrk2 R1628P as a risk factor for Parkinson's disease. *Ann. Neurol.* **64**, 88-92.
36. Healy, D. G., Falchi, M., O'Sullivan, S. S., Bonifati, V., Durr, A., Bressman, S., Brice, A., Aasly, J., Zabetian, C. P., Goldwurm, S., Ferreira, J. J., Tolosa, E., Kay, D. M., Klein, C., Williams, D. R., Marras, C., Lang, A. E., Wszolek, Z. K., Berciano, J., Schapira, A. H., Lynch, T., Bhatia, K. P., Gasser, T., Lees, A. J. and Wood, N. W. (2008) Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. *Lancet Neurol.* **7**, 583-590.
37. Ozelius, L. J., Senthil, G., Saunders-Pullman, R., Ohmann, E., Deligtisch, A., Tagliati, M., Hunt, A. L., Klein, C., Henick, B., Hailpern, S. M., Lipton, R. B., Soto-Valencia, J., Risch, N. and Bressman, S. B. (2006) LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. *N. Engl. J. Med.* **354**, 424-425.
38. Saunders-Pullman, R., Lipton, R. B., Senthil, G., Katz, M., Costan-Toth, C., Derby, C., Bressman, S., Verghese, J. and Ozelius, L. J. (2006) Increased frequency of the LRRK2 G2019S mutation in an elderly Ashkenazi Jewish population is not associated with dementia. *Neurosci. Lett.* **402**, 92-96.
39. Lesage, S., Durr, A., Tazir, M., Lohmann, E., Leutenegger, A. L., Janin, S., Pollak, P. and Brice, A. (2006) LRRK2 G2019S as a cause of Parkinson's disease in North African Arabs. *N. Engl. J. Med.* **354**, 422-423.
40. Hulihan, M. M., Ishihara-Paul, L., Kachergus, J., Warren, L., Amouri, R., Elango, R., Prinjha, R. K., Upmanyu, R., Kefi, M., Zouari, M., Sassi, S. B., Yahmed, S. B., El Euch-Fayeche, G., Matthews, P. M., Middleton, L. T., Gibson, R. A., Hentati, F. and Farrer, M. J. (2008) LRRK2 Gly2019Ser penetrance in Arab-Berber patients from Tunisia: a case-control genetic study. *Lancet Neurol.* **7**, 591-594.
41. Lesage, S., Patin, E., Condroyer, C., Leutenegger, A. L., Lohmann, E., Giladi, N., Bar-Shira, A., Belarbi, S., Hecham, N., Pollak, P., Ouvrard-Hernandez, A. M., Bardien, S., Carr, J., Benhassine, T., Tomiyama, H., Pirkevi, C., Hamadouche, T., Cazeneuve, C., Basak, A. N., Hattori, N., Durr, A., Tazir, M., Orr-Urtreger, A., Quintana-Murci, L. and Brice, A. (2010) Parkinson's disease-related LRRK2 G2019S mutation results from independent mutational events in humans. *Hum. Mol. Genet.* doi:10.1093/hmg/ddq081
42. Tan, E. K., Tan, L. C., Lim, H. Q., Li, R., Tang, M., Yih, Y., Pavanni, R., Prakash, K. M., Fook-Chong, S. and Zhao, Y. (2008) LRRK2 R1628P increases risk of Parkinson's disease: replication evidence. *Hum. Genet.* **124**, 287-288.
43. Mata, I. F., Kachergus, J. M., Taylor, J. P., Lincoln, S., Aasly, J., Lynch, T., Hulihan, M. M., Cobb, S. A., Wu, R. M., Lu, C. S., Lahoz, C., Wszolek, Z. K. and Farrer, M. J. (2005) Lrrk2 pathogenic substitutions in Parkinson's disease. *Neurogenetics* **6**, 171-177.
44. Kay, D. M., Kramer, P., Higgins, D., Zabetian, C. P. and Payami, H. (2005) Escaping Parkinson's disease: a neurologically healthy octogenarian with the LRRK2 G2019S

- mutation. *Mov. Disord.* **20**, 1077-1078.
45. Golub, Y., Berg, D., Calne, D. B., Pfeiffer, R. F., Uitti, R. J., Stoessl, A. J., Wszolek, Z. K., Farrer, M. J., Mueller, J. C., Gasser, T. and Fuchs, J. (2009) Genetic factors influencing age at onset in LRRK2-linked Parkinson disease. *Parkinsonism Relat. Disord.* **15**, 539-541.
46. Haugarvoll, K., Rademakers, R., Kachergus, J. M., Nuytemans, K., Ross, O. A., Gibson, J. M., Tan, E. K., Gaig, C., Tolosa, E., Goldwurm, S., Guidi, M., Riboldazzi, G., Brown, L., Walter, U., Benecke, R., Berg, D., Gasser, T., Theuns, J., Pals, P., Cras, P., De Deyn, P. P., Engelborghs, S., Pickut, B., Uitti, R. J., Foroud, T., Nichols, W. C., Hagenah, J., Klein, C., Samii, A., Zabetian, C. P., Bonifati, V., Van Broeckhoven, C., Farrer, M. J. and Wszolek, Z. K. (2008) Lrrk2 R1441C parkinsonism is clinically similar to sporadic Parkinson disease. *Neurology* **70**, 1456-1460.
47. Jaleel, M., Nichols, R. J., Deak, M., Campbell, D. G., Gillardon, F., Knebel, A. and Alessi, D. R. (2007) LRRK2 phosphorylates moesin at threonine-558: characterization of how Parkinson's disease mutants affect kinase activity. *Biochem. J.* **405**, 307-317.
48. West, A. B., Moore, D. J., Choi, C., Andrabi, S. A., Li, X., Dikeman, D., Biskup, S., Zhang, Z., Lim, K. L., Dawson, V. L. and Dawson, T. M. (2007) Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity. *Hum. Mol. Genet.* **16**, 223-232.
49. MacLeod, D., Dowman, J., Hammond, R., Leete, T., Inoue, K. and Abeliovich, A. (2006) The familial Parkinsonism gene LRRK2 regulates neurite process morphology. *Neuron* **52**, 587-593.
50. Melrose, H. (2008) Update on the functional biology of Lrrk2. *Future Neurol.* **3**, 669-681.
51. Greggio, E. and Cookson, M. R. (2009) Leucine-rich repeat kinase 2 mutations and Parkinson's disease: three questions. *ASN Neuro.* **1**, e00002.
52. Bretscher, A., Edwards, K. and Fehon, R. G. (2002) ERM proteins and merlin: integrators at the cell cortex. *Nat. Rev. Mol. Cell. Biol.* **3**, 586-599.
53. Parisiadou, L., Xie, C., Cho, H. J., Lin, X., Gu, X. L., Long, C. X., Lobbstaal, E., Baekelandt, V., Taymans, J. M., Sun, L. and Cai, H. (2009) Phosphorylation of ezrin/radixin/moesin proteins by LRRK2 promotes the rearrangement of actin cytoskeleton in neuronal morphogenesis. *J. Neurosci.* **29**, 13971-13980.
54. Gillardon, F. (2009) Leucine-rich repeat kinase 2 phosphorylates brain tubulin-beta isoforms and modulates microtubule stability - a point of convergence in Parkinsonian neurodegeneration? *J. Neurochem.* **110**, 1514-1522.
55. Imai, Y., Gehrke, S., Wang, H. Q., Takahashi, R., Hasegawa, K., Oota, E. and Lu, B. (2008) Phosphorylation of 4E-BP by LRRK2 affects the maintenance of dopaminergic neurons in *Drosophila*. *EMBO J.* **27**, 2432-2443.
56. Tain, L. S., Mortiboys, H., Tao, R. N., Ziviani, E., Bandmann, O. and Whitworth, A. J. (2009) Rapamycin activation of 4E-BP prevents parkinsonian dopaminergic neuron loss. *Nat. Neurosci.* **12**, 1129-1135.
57. Kumar, A., Greggio, E., Beilina, A., Kaganovich, A., Chan, D., Taymans, J. M., Wolozin, B. and Cookson, M. R. (2010) The Parkinson's disease associated LRRK2 exhibits weaker in vitro phosphorylation of 4E-BP compared to autophosphorylation. *PLoS One* **5**, e8730.
58. Gloeckner, C. J., Schumacher, A., Boldt, K. and Ueffing, M. (2009) The Parkinson disease-associated protein kinase LRRK2 exhibits MAPKKK activity and phosphorylates MKK3/6 and MKK4/7, in vitro. *J. Neurochem.* **109**, 959-968.
59. Hsu, C. H., Chan, D., Greggio, E., Saha, S., Guillily, M. D., Ferree, A., Raghavan, K., Shen, G. C., Segal, L., Ryu, H., Cookson, M. R. and Wolozin, B. (2010) MKK6 binds and regulates expression of Parkinson's disease-related protein LRRK2. *J. Neurochem.* **112**, 1593-1604.
60. Kamikawaji, S., Ito, G. and Iwatsubo, T. (2009) Identification of the autophosphorylation sites of LRRK2. *Biochemistry* **48**, 10963-10975.
61. Nichols, R. J., Dzamko, N., Hutti, J. E., Cantley, L. C., Deak, M., Moran, J., Bamorough, P., Reith, A. D. and Alessi, D. R. (2009) Substrate specificity and inhibitors of LRRK2, a protein kinase mutated in Parkinson's disease. *Biochem. J.* **424**, 47-60.
62. Narumiya, S. (1996) The small GTPase Rho: cellular functions and signal transduction. *J. Biochem.* **120**, 215-228.
63. Li, X., Tan, Y. C., Poulouse, S., Olanow, C. W., Huang, X. Y. and Yue, Z. (2007) Leucine-rich repeat kinase 2 (LRRK2)/PARK8 possesses GTPase activity that is altered in familial Parkinson's disease R1441C/G mutants. *J. Neurochem.* **103**, 238-247.
64. Guo, L., Gandhi, P. N., Wang, W., Petersen, R. B., Wilson-Delfosse, A. L. and Chen, S. G. (2007) The Parkinson's disease-associated protein, leucine-rich repeat kinase 2 (LRRK2), is an authentic GTPase that stimulates kinase activity. *Exp. Cell Res.* **313**, 3658-3670.
65. Lewis, P. A., Greggio, E., Beilina, A., Jain, S., Baker, A. and Cookson, M. R. (2007) The R1441C mutation of LRRK2 disrupts GTP hydrolysis. *Biochem. Biophys. Res. Commun.* **357**, 668-671.
66. Deng, J., Lewis, P. A., Greggio, E., Sluch, E., Beilina, A. and Cookson, M. R. (2008) Structure of the ROC domain from the Parkinson's disease-associated leucine-rich repeat kinase 2 reveals a dimeric GTPase. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 1499-1504.
67. Ito, G., Okai, T., Fujino, G., Takeda, K., Ichijo, H., Kataoka, T. and Iwatsubo, T. (2007) GTP binding is essential to the protein kinase activity of LRRK2, a causative gene product for familial Parkinson's disease. *Biochemistry* **46**, 1380-1388.
68. Liu, M., Dobson, B., Glicksman, M. A., Yue, Z. and Stein, R. L. (2010) Kinetic mechanistic studies of wild-type leucine-rich repeat kinase2: characterization of the kinase and GTPase activities. *Biochemistry* **49**, 2008-2017.
69. Greggio, E., Jain, S., Kingsbury, A., Bandopadhyay, R., Lewis, P., Kaganovich, A., van der Brug, M. P., Beilina, A., Blackinton, J., Thomas, K. J., Ahmad, R., Miller, D. W., Kesavapany, S., Singleton, A., Lees, A., Harvey, R. J., Harvey, K. and Cookson, M. R. (2006) Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. *Neurobiol. Dis.* **23**, 329-341.
70. Gasper, R., Meyer, S., Gotthardt, K., Sirajuddin, M. and

- Wittinghofer, A. (2009) It takes two to tango: regulation of G proteins by dimerization. *Nat. Rev. Mol. Cell Biol.* **10**, 423-429.
71. Sen, S., Webber, P. J. and West, A. B. (2009) Dependence of leucine-rich repeat kinase 2 (LRRK2) kinase activity on dimerization. *J. Biol. Chem.* **284**, 36346-36356.
72. Greggio, E., Zambrano, I., Kaganovich, A., Beilina, A., Taymans, J. M., Daniels, V., Lewis, P., Jain, S., Ding, J., Syed, A., Thomas, K. J., Baekelandt, V. and Cookson, M. R. (2008) The Parkinson disease-associated leucine-rich repeat kinase 2 (LRRK2) is a dimer that undergoes intramolecular autophosphorylation. *J. Biol. Chem.* **283**, 16906-16914.
73. Gandhi, P. N., Wang, X., Zhu, X., Chen, S. G. and Wilson-Delfosse, A. L. (2008) The Roc domain of leucine-rich repeat kinase 2 is sufficient for interaction with microtubules. *J. Neurosci. Res.* **86**, 1711-1720.
74. Dachsel, J. C., Taylor, J. P., Mok, S. S., Ross, O. A., Hinkle, K. M., Bailey, R. M., Hines, J. H., Szutu, J., Madden, B., Petrucelli, L. and Farrer, M. J. (2007) Identification of potential protein interactors of Lrrk2. *Parkinsonism Relat. Disord.* **13**, 382-385.
75. Wang, L., Xie, C., Greggio, E., Parisiadou, L., Shim, H., Sun, L., Chandran, J., Lin, X., Lai, C., Yang, W. J., Moore, D. J., Dawson, T. M., Dawson, V. L., Chiosis, G., Cookson, M. R. and Cai, H. (2008) The chaperone activity of heat shock protein 90 is critical for maintaining the stability of leucine-rich repeat kinase 2. *J. Neurosci.* **28**, 3384-3391.
76. Ko, H. S., Bailey, R., Smith, W. W., Liu, Z., Shin, J. H., Lee, Y. I., Zhang, Y. J., Jiang, H., Ross, C. A., Moore, D. J., Patterson, C., Petrucelli, L., Dawson, T. M. and Dawson, V. L. (2009) CHIP regulates leucine-rich repeat kinase-2 ubiquitination, degradation, and toxicity. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 2897-2902.
77. Ding, X. and Goldberg, M. S. (2009) Regulation of LRRK2 stability by the E3 ubiquitin ligase CHIP. *PLoS One* **4**, e5949.
78. Ohta, E., Katayama, Y., Kawakami, F., Yamamoto, M., Tajima, K., Maekawa, T., Iida, N., Hattori, S. and Obata, F. (2009) I2020T leucine-rich repeat kinase 2, the causative mutant molecule of familial Parkinson's disease, has a higher intracellular degradation rate than the wild-type molecule. *Biochem. Biophys. Res. Commun.* **390**, 710-715.
79. Ohta, E., Kubo, M. and Obata, F. (2010) Prevention of intracellular degradation of I2020T mutant LRRK2 restores its protectivity against apoptosis. *Biochem. Biophys. Res. Commun.* **391**, 242-247.
80. Ho, C. C., Rideout, H. J., Ribe, E., Troy, C. M. and Dauer, W. T. (2009) The Parkinson disease protein leucine-rich repeat kinase 2 transduces death signals via Fas-associated protein with death domain and caspase-8 in a cellular model of neurodegeneration. *J. Neurosci.* **29**, 1011-1016.
81. Sancho, R. M., Law, B. M. and Harvey, K. (2009) Mutations in the LRRK2 Roc-COR tandem domain link Parkinson's disease to Wnt signalling pathways. *Hum. Mol. Genet.* **18**, 3955-3968.
82. Smith, W. W., Pei, Z., Jiang, H., Moore, D. J., Liang, Y., West, A. B., Dawson, V. L., Dawson, T. M. and Ross, C. A. (2005) Leucine-rich repeat kinase 2 (LRRK2) interacts with parkin, and mutant LRRK2 induces neuronal degeneration. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 18676-18681.
83. Venderova, K., Kabbach, G., Abdel-Messih, E., Zhang, Y., Parks, R. J., Imai, Y., Gehrke, S., Ngsee, J., Lavoie, M. J., Slack, R. S., Rao, Y., Zhang, Z., Lu, B., Haque, M. E. and Park, D. S. (2009) Leucine-Rich Repeat Kinase 2 interacts with Parkin, DJ-1 and PINK-1 in a Drosophila melanogaster model of Parkinson's disease. *Hum. Mol. Genet.* **18**, 4390-4404.
84. Samann, J., Hegermann, J., von Gromoff, E., Eimer, S., Baumeister, R. and Schmidt, E. (2009) Caenorhabditis elegans LRK-1 and PINK-1 act antagonistically in stress response and neurite outgrowth. *J. Biol. Chem.* **284**, 16482-16491.
85. Lin, X., Parisiadou, L., Gu, X. L., Wang, L., Shim, H., Sun, L., Xie, C., Long, C. X., Yang, W. J., Ding, J., Chen, Z. Z., Gallant, P. E., Tao-Cheng, J. H., Rudow, G., Troncoso, J. C., Liu, Z., Li, Z. and Cai, H. (2009) Leucine-rich repeat kinase 2 regulates the progression of neuro pathology induced by Parkinson's-disease-related mutant alpha-synuclein. *Neuron* **64**, 807-827.
86. Ng, C. H., Mok, S. Z., Koh, C., Ouyang, X., Fivaz, M. L., Tan, E. K., Dawson, V. L., Dawson, T. M., Yu, F. and Lim, K. L. (2009) Parkin protects against LRRK2 G2019S mutant-induced dopaminergic neurodegeneration in Drosophila. *J. Neurosci.* **29**, 11257-11162.
87. Plowey, E. D., Cherra, S. J., 3rd, Liu, Y. J. and Chu, C. T. (2008) Role of autophagy in G2019S-LRRK2-associated neurite shortening in differentiated SH-SY5Y cells. *J. Neurochem.* **105**, 1048-1056.
88. Liou, A. K., Leak, R. K., Li, L. and Zigmund, M. J. (2008) Wild-type LRRK2 but not its mutant attenuates stress-induced cell death via ERK pathway. *Neurobiol. Dis.* **32**, 116-124.
89. Heo, H. Y., Park, J. M., Kim, C. H., Han, B. S., Kim, K. S. and Seol, W. (2010) LRRK2 enhances oxidative stress-induced neurotoxicity via its kinase activity. *Exp. Cell Res.* **316**, 649-656.
90. Alegre-Abarrategui, J., Christian, H., Lufino, M., Mutihac, R., Lourenco Venda, L., Ansorge, O. and Wade-Martins, R. (2009) LRRK2 regulates autophagic activity and localises to specific membrane microdomains in a novel human genomic reporter cellular model. *Hum. Mol. Genet.* **18**, 4022-4033.
91. Iaccarino, C., Crosio, C., Vitale, C., Sanna, G., Carri, M. T. and Barone, P. (2007) Apoptotic mechanisms in mutant LRRK2-mediated cell death. *Hum. Mol. Genet.* **16**, 1319-1326.
92. Carballo-Carbajal, I., Weber-Endress, S., Rovelli, G., Chan, D., Wolozin, B., Klein, C. L., Patenge, N., Gasser, T. and Kahle, P. J. (2010) Leucine-rich repeat kinase 2 induces alpha-synuclein expression via the extracellular signal-regulated kinase pathway. *Cell Signal* **22**, 821-827.
93. Li, Y., Liu, W., Oo, T. F., Wang, L., Tang, Y., Jackson-Lewis, V., Zhou, C., Goghman, K., Bogdanov, M., Przedborski, S., Beal, M. F., Burke, R. E. and Li, C. (2009) Mutant LRRK2 (R1441G) BAC transgenic mice recapitulate

- tulate cardinal features of Parkinson's disease. *Nat. Neurosci.* **12**, 826-828.
94. Tong, Y., Pisani, A., Martella, G., Karouani, M., Yamaguchi, H., Pothos, E. N. and Shen, J. (2009) R1441C mutation in LRRK2 impairs dopaminergic neurotransmission in mice. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 14622-14627.
95. Li, X., Patel, J. C., Wang, J., Avshalumov, M. V., Nicholson, C., Buxbaum, J. D., Elder, G. A., Rice, M. E. and Yue, Z. (2010) Enhanced striatal dopamine transmission and motor performance with LRRK2 overexpression in mice is eliminated by familial Parkinson's disease mutation G2019S. *J. Neurosci.* **30**, 1788-1797.
96. Saha, S., Guillily, M. D., Ferree, A., Lanceta, J., Chan, D., Ghosh, J., Hsu, C. H., Segal, L., Raghavan, K., Matsumoto, K., Hisamoto, N., Kuwahara, T., Iwatsubo, T., Moore, L., Goldstein, L., Cookson, M. and Wolozin, B. (2009) LRRK2 modulates vulnerability to mitochondrial dysfunction in *Caenorhabditis elegans*. *J. Neurosci.* **29**, 9210-9218.
97. Wang, D., Tang, B., Zhao, G., Pan, Q., Xia, K., Bodmer, R. and Zhang, Z. (2008) Dispensable role of *Drosophila* ortholog of LRRK2 kinase activity in survival of dopaminergic neurons. *Mol. Neurodegener.* **3**, 3.
98. Liu, Z., Wang, X., Yu, Y., Li, X., Wang, T., Jiang, H., Ren, Q., Jiao, Y., Sawa, A., Moran, T. Ross, C. A., Montell, C. and Smith, W. W. (2008) A *Drosophila* model for LRRK2-linked parkinsonism. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 2693-2698.
99. White, L. R., Toft, M., Kvam, S. N., Farrer, M. J. and Aasly, J. O. (2007) MAPK-pathway activity, Lrrk2 G2019S, and Parkinson's disease. *J. Neurosci. Res.* **85**, 1288-1294.
100. Mutez, E., Larvor, L., Lepretre, F., Mouroux, V., Hamalek, D., Kerckaert, J. P., Perez-Tur, J., Waucquier, N., Vanbesien-Mailliot, C., Dufloy, A., Devos, D., Defebvre, L., Kreisler, A., Frigard, B., Destee, A. and Chartier-Harlin, M. C. (2010) Transcriptional profile of Parkinson blood mononuclear cells with LRRK2 mutation. *Neurobiol. Aging*. doi:10.1016/j.neurobiolaging.2009.10.016
101. Johansen, K. K., Wang, L., Aasly, J. O., White, L. R., Mattson, W. R., Henchcliffe, C., Beal, M. F. and Bogdanov, M. (2009) Metabolomic profiling in LRRK2-related Parkinson's disease. *PLoS One* **4**, e7551.
102. Church, W. H. and Ward, V. L. (1994) Uric acid is reduced in the substantia nigra in Parkinson's disease: effect on dopamine oxidation. *Brain Res. Bull.* **33**, 419-425.
103. Covy, J. P. and Giasson, B. I. (2009) Identification of compounds that inhibit the kinase activity of leucine-rich repeat kinase 2. *Biochem. Biophys. Res. Commun.* **378**, 473-477.
104. Yue, Z. (2009) LRRK2 in Parkinson's disease: *in vivo* models and approaches for understanding pathogenic roles. *FEBS J.* **276**, 6445-6454.
105. Webber, P. J. and West, A. B. (2009) LRRK2 in Parkinson's disease: function in cells and neurodegeneration. *FEBS J.* **276**, 6436-6444.
106. Kumari, U. and Tan, E. K. (2009) LRRK2 in Parkinson's disease: genetic and clinical studies from patients. *FEBS J.* **276**, 6455-6463.
107. Braithwaite, S. P. (2009) LRRK2 in Parkinson's disease: building an understanding of disease etiology. *FEBS J.* **276**, 6427.
108. Anand, V. S. and Braithwaite, S. P. (2009) LRRK2 in Parkinson's disease: biochemical functions. *FEBS J.* **276**, 6428-6435.
109. Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Di Iorio, G., Golbe, L. I. and Nussbaum, R. L. (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* **276**, 2045-2047.
110. Gasser, T., Muller-Myhsok, B., Wszolek, Z. K., Oehlmann, R., Calne, D. B., Bonifati, V., Bereznoi, B., Fabrizio, E., Vieregge, P. and Horstmann, R. D. (1998) A susceptibility locus for Parkinson's disease maps to chromosome 2p13. *Nat. Genet.* **18**, 262-265.
111. Leroy, E., Boyer, R., Auburger, G., Leube, B., Ulm, G., Mezey, E., Harta, G., Brownstein, M. J., Jonnalagada, S., Chernova, T., Dehejia, A., Lavedan, C., Gasser, T., Steinbach, P. J., Wilkinson, K. D. and Polymeropoulos, M. H. (1998) The ubiquitin pathway in Parkinson's disease. *Nature* **395**, 451-452.
112. Valente, E. M., Abou-Sleiman, P. M., Caputo, V., Muqit, M. M., Harvey, K., Gispert, S., Ali, Z., Del Turco, D., Bentivoglio, A. R., Healy, D. G., Albanese, A., Nussbaum, R., Gonzalez-Maldonado, R., Deller, T., Salvi, S., Cortelli, P., Gilks, W. P., Latchman, D. S., Harvey, R. J., Dallapiccola, B., Auburger, G. and Wood, N. W. (2004) Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* **304**, 1158-1160.
113. Bonifati, V., Rizzu, P., van Baren, M. J., Schaap, O., Breedveld, G. J., Krieger, E., Dekker, M. C., Squitieri, F., Ibanez, P., Joosse, M. van Dongen, J. W., Vanacore, N., van Swieten, J. C., Brice, A., Meco, G., van Duijn, C. M., Oostra, B. A. and Heutink, P. (2003) Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science* **299**, 256-259.
114. Ramirez, A., Heimbach, A., Grundemann, J., Stiller, B., Hampshire, D., Cid, L. P., Goebel, I., Mubaidin, A. F., Wriekat, A. L., Roeper, J. Al-Din, A., Hillmer, A. M., Karsak, M., Liss, B., Woods, C. G., Behrens, M. I. and Kubisch, C. (2006) Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. *Nat. Genet.* **38**, 1184-1191.
115. Li, Y. J., Scott, W. K., Hedges, D. J., Zhang, F., Gaskell, P. C., Nance, M. A., Watts, R. L., Hubble, J. P., Koller, W. C., Pahwa, R., Stern, M. B., Hiner, B. C., Jankovic, J., Allen, F. A., Jr., Goetz, C. G., Mastaglia, F., Stajich, J. M., Gibson, R. A., Middleton, L. T., Saunders, A. M., Scott, B. L., Small, G. W., Nicodemus, K. K., Reed, A. D., Schmechel, D. E., Welsh-Bohmer, K. A., Conneally, P. M., Roses, A. D., Gilbert, J. R., Vance, J. M., Haines, J. L. and Pericak-Vance, M. A. (2002) Age at onset in two common neurodegenerative diseases is genetically controlled. *Am. J. Hum. Genet.* **70**, 985-993.

116. Lautier, C., Goldwurm, S., Durr, A., Giovannone, B., Tsiaras, W. G., Pezzoli, G., Brice, A. and Smith, R. J. (2008) Mutations in the GIGYF2 (TNRC15) gene at the PARK11 locus in familial Parkinson disease. *Am. J. Hum. Genet.* **82**, 822-833.
117. Pankratz, N., Nichols, W. C., Uniacke, S. K., Halter, C., Rudolph, A., Shults, C., Conneally, P. M. and Foroud, T. (2002) Genome screen to identify susceptibility genes for Parkinson disease in a sample without parkin mutations. *Am. J. Hum. Genet.* **71**, 124-135.
118. Strauss, K. M., Martins, L. M., Plun-Favreau, H., Marx, F. P., Kautzmann, S., Berg, D., Gasser, T., Wszolek, Z., Muller, T., Bornemann, A., Wolburg, H., Downward, J., Riess, O., Schulz, J. B. and Kruger, R. (2005) Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. *Hum. Mol. Genet.* **14**, 2099-2111.
119. Paisan-Ruiz, C., Bhatia, K. P., Li, A., Hernandez, D., Davis, M., Wood, N. W., Hardy, J., Houlden, H., Singleton, A. and Schneider, S. A. (2009) Characterization of PLA2G6 as a locus for dystonia-parkinsonism. *Ann. Neurol.* **65**, 19-23.
120. Shojaei, S., Sina, F., Banihosseini, S. S., Kazemi, M. H., Kalhor, R., Shahidi, G. A., Fakhrai-Rad, H., Ronaghi, M. and Elahi, E. (2008) Genome-wide linkage analysis of a Parkinsonian-pyramidal syndrome pedigree by 500 K SNP arrays. *Am. J. Hum. Genet.* **82**, 1375-1384.
121. Di Fonzo, A., Dekker, M. C., Montagna, P., Baruzzi, A., Yonova, E. H., Correia Guedes, L., Szczerbinska, A., Zhao, T., Dubbel-Hulsman, L. O., Wouters, C. H. de Graaff, E., Oyen, W. J., Simons, E. J., Breedveld, G. J., Oostra, B. A., Horstink, M. W. and Bonifati, V. (2009) FBXO7 mutations cause autosomal recessive, early-onset parkinsonian-pyramidal syndrome. *Neurology* **72**, 240-245.
122. Healy, D. G., Abou-Sleiman, P. M., Casas, J. P., Ahmadi, K. R., Lynch, T., Gandhi, S., Muqit, M. M., Foltynie, T., Barker, R., Bhatia, K. P., Quinn, N. P., Lees, A. J., Gibson, J. M., Holton, J. L., Revesz, T., Goldstein, D. B. and Wood, N. W. (2006) UCHL-1 is not a Parkinson's disease susceptibility gene. *Ann. Neurol.* **59**, 627-633.
123. Nichols, W. C., Kissell, D. K., Pankratz, N., Pauciuolo, M. W., Elsaesser, V. E., Clark, K. A., Halter, C. A., Rudolph, A., Wojcieszek, J., Pfeiffer, R.F. and Foroud, T. (2009) Variation in GIGYF2 is not associated with Parkinson disease. *Neurology* **72**, 1886-1892.
124. Vilarino-Guell, C., Ross, O. A., Soto, A. I., Farrer, M. J., Haugavoll, K., Aasly, J. O., Uitti, R. J. and Wszolek, Z. K. (2009) Reported mutations in GIGYF2 are not a common cause of Parkinson's disease. *Mov. Disord.* **24**, 619-620.