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Measuring the Activity of Leucine-Rich Repeat Kinase 2: A Kinase Involved in Parkinson's Disease

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Abstract

Mutations in the *LRRK2* (Leucine-Rich Repeat Kinase 2) gene are the most common cause of autosomal dominant Parkinson's disease. *LRRK2* has multiple functional domains including a kinase domain. The kinase activity of *LRRK2* is implicated in the pathogenesis of Parkinson's disease. Developing an assay to understand the mechanisms of *LRRK2* kinase activity is important for the development of pharmacologic and therapeutic applications. Here, we describe how to measure in vitro *LRRK2* kinase activity and its inhibition.

Keywords

Parkinson's disease; *LRRK2*; In vitro kinase assay

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease in the world. It affects approximately 1–2% of the US population above the age of 65. The pathological hallmark of PD is the presence of cytoplasmic inclusions known as Lewy bodies and the degeneration of dopaminergic neurons in the nigrostriatal pathway (1). Although the majority of PD cases are sporadic, there are familial cases of PD in which mutations in a variety of genes have been linked to PD. This suggests an unambiguous role of genetic component in the development of PD (2, 3). Previous studies provide us various clues for the pathogenesis of PD. The identification of the *Leucine-Rich Repeat Kinase 2* (*LRRK2*)-linked PD has opened up new opportunities for the study of etiology of PD and the discovery of novel therapeutic targets for PD.

LRRK2 mutations are very common in Parkinson's disease (PD) patients, both sporadic and familial. *LRRK2*-linked PD patients showed very similar clinical and neuropathologic features as idiopathic PD (4, 5). Familial mutations within the *LRRK2* gene have been found to cause the alterations of amino acid throughout the entire *LRRK2* protein (5–11). *LRRK2* contains multiple functional domains, including two enzymatic domains (GTPase domain and kinase domain) and two protein–protein interacting domains (N-terminal LRR domain

and C-terminal WD40 domain) (12). The exact physiological function of LRRK2 protein is still not yet clear. However, genetic and biochemical studies suggest that LRRK2 mutations most likely cause disease through a dominant gain-of-function mechanism. Many disease-associated LRRK2 mutant proteins showed enhanced kinase activity in the in vitro kinase assay. Expression of LRRK2 protein can lead to cytotoxicity in cultured cells and primary neurons. Toxicity is dependent on kinase activity and GTP binding activity of LRRK2 (13–16). To further study kinase activity and toxicity of LRRK2 protein, an optimized in vitro kinase assay was developed. We show that exogenously overexpressed LRRK2 protein can autophosphorylate itself and the generic substrate, myelin basic protein (MBP).

2. Materials

2.1. Cell Culture

1. Human Embryonic Kidney (HEK) 293 FT cells.
2. Opti-MEM I Reduced–Serum Media (1×) liquid.
3. Fetal bovine serum.
4. TrypLE™ Express with Phenol Red.
5. Fugene HD Transfection Reagent.
6. LRRK2: a plasmid containing GST-tagged LRRK2 is available from the authors on request.

2.2. Cell Lysis and Preparation of LRRK2

1. Phosphate-buffered Saline (PBS, 10× stock): 1.37 mM NaCl, 27 mM KCl, 100 mM Na₂HPO₄, and 18 mM KH₂PO₄. Adjust to pH 7.4 with HCl.
2. Cell lysis buffer: 50 mM HEPES, 150 mM NaCl, 5 mM EGTA, 0.5% NP-40. Adjust to pH 7.4 with HCl.
3. Washing buffer: 1× PBS with 0.5% NP-40 and 150 mM NaCl.
4. Gluthathione-Sepharose 4B.
5. Protein G Sepharose, Protein A Sepharose, or protein G Dynabead (see Note 1).

2.3. In Vitro Kinase assay

1. Kinase assay buffer: 20 mM HEPES, 150 mM NaCl, 5 mM EGTA, and 20 mM b-Glycerol phosphate. Adjust pH to 7.4. Make 2 M b-glycerol phosphate and freeze single-use aliquots (500 µl) at –20°C.
2. MgCl₂ (2 M stock): Store at room temperature.
3. Adenosine 5'-triphosphate disodium salt (ATP, 99% purity, 100 mM stock): freeze single-use aliquots (10 µl) at –80°C.

¹Protein A sepharose can also be used if your antibody comes from rabbit (total Ig) or mouse (total Ig) serum (see Subheading 2.2).

4. Adenosine 5'-triphosphate, [g-³²P]: 6,000 Ci/mmol 10 mCi/ml EasyTide. Store at 4°C (see Note 2).
5. Myelin Basic Protein (MBP, 5 µg/µl stock): The assay described in this protocol used MBP, dephosphorylated, produced from bovine brain. Freeze single-use aliquots (20 µl) at −20°C (see Note 3).
6. Laemmli sample buffer (5× stock): 0.25 M Tris-HCl, pH 6.8, 6% (w/v) SDS, 40% (w/v) glycerol, 0.04% (w/v) bromophenol blue, 12.5% (v/v) β-mercaptoethanol. Freeze in 1 ml aliquots at −20°C.

2.4. SDS-Polyacrylamide Gel Electrophoresis

1. Separate gel buffer (4× stock): 1.5 M Tris-HCl, pH 8.8, 0.4% SDS.
2. Stacking gel buffer (4× stock): 0.5 M Tris-HCl, pH 6.8, 0.4% SDS.
3. 30% acrylamide/bisacrylamide solution (see Note 4).
4. N,N,N',N'-tetramethyl-ethylenediamine (TEMED).
5. Ammonium persulfate: 10% (w/v) solution in water. Freeze single-use aliquots (200 µl) at −20°C.
6. Isobutanol.
7. Precision plus protein dual-color standards.
8. Running buffer (10× stock): 250 mM Tris (do not adjust pH), 1.92 M glycine, 1% (w/v) SDS.

2.5. Coomassie Brilliant Staining

1. Fixing solution: 10% (v/v) acetic acid, 40% (v/v) methanol in water. Prepare fresh solution every time.
2. Colloidal Blue Staining Kit: any such kit may be used.

2.6. Western Blotting for LRRK2

1. Transfer buffer (10× stock): 250 mM Tris (do not adjust pH), 1.92 M glycine. Prepare working solution by diluting one part of stock with seven parts water and adding two parts of methanol.
2. Nitrocellulose membrane and 3 MM chromatography paper.
3. Tris-buffered saline with Tween20 (TBST, 10× stock): 200 mM Tris-HCl, pH 7.4, 1.37 M NaCl, 0.5% Tween20.
4. Blocking buffer: 5% (w/v) nonfat dry milk in 1× TBST.

²The physical half-life of ³²P is 14.3 days. Use isotope within 1 month after manufacture for kinase assay. Take all precautions required by your local authorities when working with radioactive isotopes (see Subheading 2.3).

³Because purified MBP is usually basally phosphorylated, dephosphorylated MBP (commercially available) is better to use as a kinase substrate in the kinase assay (see Subheading 2.3).

⁴Acrylamide is neurotoxic when unpolymerized, so care should be taken (see Subheading 2.4).

5. Antibody dilution buffer: 5% (w/v) nonfat dry milk in 1× TBST.
6. Enhanced chemiluminescent (ECL) reagent and X-ray film.
7. Horseradish peroxidase (HRP) conjugated anti-GST antibody.

2.7. Radiography

1. Storage Phosphor Screen and Cassette.
2. Phosphoimager: Typhoon 9410 and ImageQuant 6.0 software.

3. Methods

LRRK2 encodes a kinase domain with highest similarity to the mixed lineage kinase (MLK) motif found in proteins that commonly have both Ser/Thr and Tyr kinase activities. The most convenient method to detect the active form of the kinase is using the phosphospecific antibodies, such as phospho-p38 MAPK (Thr180/Tyr182) and phospho-AKT (S473) antibodies. However, because the phosphospecific antibody that detects active LRRK2 is not available yet, West et al. (17) developed an assay that measures the ability of LRRK2 to phosphorylate generic substrate of kinases, myelin basic protein (MBP) by measuring the incorporation of [^{32}P] radioisotope-labeled phosphate to the kinase substrates. This assay also detects autophosphorylation of recombinant LRRK2 in the absence of any potential cofactor or activators (17). Here, we describe how to prepare recombinant LRRK2 and how to measure its activity by checking its autophosphorylation and MBP phosphorylation.

Because of technical difficulty in expressing LRRK2 protein, sufficient amount of the protein cannot be prepared. Currently, the most effective method for preparation of full length LRRK2 protein is by using mammalian expression systems. Several epitope-tagged LRRK2 proteins have been developed including myc-LRRK2 (17), V5-LRRK2 (13), HA-LRRK2 (18), flag-LRRK2 (14), and GST-LRRK2 (19). By using these epitope-tagged LRRK2 systems, recombinant LRRK2 protein can be prepared after transiently expression in HEK293 cells. Prepared recombinant LRRK2 protein is incubated with inhibitor and/or activator in the presence of [$\gamma\text{-}^{32}\text{P}$] ATP and MBP. LRRK2 autophosphorylation and MBP phosphorylation can be estimated using a Phosphoimager and suitable software (e.g., ImageQuant 6.0). Input levels of protein present on the gel are determined by coomassie brilliant blue staining.

3.1. Preparation of Recombinant LRRK2 Protein

3.1.1. Transfection for Transient Expression of LRRK2 Protein in HEK293 FT Cells

1. HEK293 FT cells are cultured in Opti-MEM I medium supplemented with 10% FBS. Cells are passaged every 3 or 4 days when approaching confluence with TrypLETM Express to new culture dishes. For the transfection, confluent cells are split into 60-mm culture dish for a single experiment data point.
2. Fugene HD is used as a transfection reagent to transiently express LRRK2 in HEK293 FT cells. Transfection is performed 1 day after plating the cells. First,

dilute 4 µg DNA to 0.2 ml Opti-MEM I without serum and then add 12 µl Eugene HD to DNA-diluted medium. Briefly vortex the transfection mixture and incubate for 15 min at room temperature. After incubation, add the transfection mixture to the cells in a drop-wise manner on the medium. Before collecting the cells, incubate for 48 h at 37°C in a CO₂ incubator.

3.1.2. Preparation of Epitope-Tagged LRRK2 Proteins from HEK293 FT Cells

1. 1 day before collecting the transfected cells, the immune complex of antibody and protein G or protein A has to be prepared. To prepare the immune complex, resuspend protein G sepharose or protein A sepharose thoroughly to obtain a homogeneous suspension. Transfer 30 µl protein G sepharose or protein A sepharose to a microcentrifuge tube. Wash with 1 ml PBS and sediment the resin by centrifugation at $1,000 \times g$ for 5 min. Discard the supernatant and repeat this wash step. Add 30 µl PBS and 1–2 µg antibody. Rotate this complex at 4°C overnight.
2. Sediment the immune complex by centrifugation at $1,000 \times g$ for 5 min. Remove the supernatant and wash the resin with 1 ml lysis buffer. Sediment the resin by centrifugation at $1,000 \times g$ for 5 min. The pellet contains the immune complex.
3. Prepare cold PBS and lysis buffer containing protease inhibitors. After aspirating the medium, add 4 ml of cold PBS to the culture dish and place culture dish on ice. After aspirating PBS, add 0.5 ml of cold lysis buffer to culture dish. Scrape cells with a cell scraper and transfer the lysate to a microcentrifuge tube. Vigorously vortex the lysate and rotate at 4°C for 30 min followed by centrifugation at $20,000 \times g$ for 15 min.
4. Take 20 µl supernatant to check LRRK2 expression. Mix the immune complex (prepared in step 1–2) and cell lysate and rotate at 4°C overnight.
5. Collect the resin by centrifugation at $1,000 \times g$ for 5 min and discard the supernatant. Resuspend the resin with 0.5 ml washing buffer and sediment the resin by centrifugation at $1,000 \times g$ for 5 min. Discard the supernatant and repeat the wash three more times.
6. After the last washing step, discard the supernatant and wash the resin with 0.5 ml kinase assay buffer. Sediment by centrifugation at $1,000 \times g$ for 5 min and add 20 µl kinase assay buffer (see Note 5).

3.1.3. Preparation of GST-Fusion LRRK2 Proteins from HEK293 FT Cells

1. Prepare cold PBS and lysis buffer containing protease inhibitors. After aspirating the medium from HEK293 FT cells transfected with GST-fusion LRRK2, add 4 ml of cold PBS to culture dish and place culture dish on ice. After aspirating PBS, add 0.5 ml of cold lysis buffer to the culture dish. Scrape the cells with a cell scraper

⁵After final washing step, supernatant has to be completely removed. Using a 1-ml syringe with a 26 gauge needle will be helpful (see Subheading 3.1.2).

and transfer to a microcentrifuge tube. Vigorously vortex the lysate and rotate at 4°C for 30 min followed by centrifugation at $20,000 \times g$ for 15 min.

2. Resuspend Glutathione-Sepharose thoroughly to obtain a homogeneous suspension. Transfer 30 μ l slurry to a microcentrifuge tube. Wash the slurry with 1 ml PBS and sediment the slurry by centrifugation at $1,000 \times g$ for 5 min. Discard the supernatant and repeat this wash step.
3. Take 20 μ l supernatant from step 1 to check LRRK2 expression. Mix the washed slurry and supernatant from step 1. Rotate the mixture at 4°C overnight.
4. Collect the resin by centrifugation at $1,000 \times g$ for 5 min and discard the supernatant. Resuspend the resin with 0.5 ml washing buffer and sediment by centrifugation at $1,000 \times g$ for 5 min. Discard the supernatant and repeat the washing step three more times (see Note 5).
5. After washing the resin, resuspend the resin with 0.5 ml of elution buffer without glutathione. After centrifugation at $1,000 \times g$ for 5 min, discard the supernatant and add 30 μ l elution buffer with 20 mM glutathione to the resin. Rotate the mixture at 4°C for 30 min. Eluted protein is obtained after centrifugation at $1,000 \times g$ for 5 min. Transfer 20 μ l of eluted LRRK2 protein to a microcentrifuge tube.

3.2. In Vitro Kinase Assay of LRRK2

1. Prepare 20 μ l of reaction volume by adding kinase assay buffer to recombinant LRRK2 protein.
2. Inhibitors or activators to be tested can be added at an appropriate concentration. The volume of inhibitor/activator must not exceed 0.5 μ l. Preincubate recombinant LRRK2 protein and inhibitor/activator mixture for 5 min at 30°C. When immunoprecipitated recombinant LRRK2 is used in the kinase assay, the reaction tube has to be stirred to prevent sedimentation of resin. An orbital mixing heating plate will be helpful. However, stirring is not necessary when using purified GST-fusion LRRK2 protein.
3. The kinase reaction is initiated with addition of 5 μ l of kinase reaction buffer containing 10 mM ATP, 20 mM $MgCl_2$, 2.5 μ g MBP, and 0.5 μ Ci [32 P] ATP after preincubation of recombinant LRRK2 protein and inhibitor/activator. Incubate the reaction for 15 min at 30°C with gentle rocking.
4. Stop the reaction by adding 6.25 μ l of 5 $\times$ Laemmli sample buffer. Heat the sample at 75°C for 10 min (see Note 6). Sediment the resin at $1,000 \times g$ for 5 min and the supernatant is resolved onto a SDS-PAGE gel.

3.3. SDS-PAGE

1. These instructions are for the Mini-PROTEAN Electrophoresis System from Bio-Rad Laboratories. Other minigel systems may also be used. Prepare two different

⁶LRRK2 may be degraded if boiled at 100°C (see Subheading 3.2).

percentages of SDS-PAGE gels. Use a 12% gel to detect phosphorylation of LRRK2 and MBP and a 6% gel to confirm LRRK2 expression.

2. Prepare a 12% separation gel solution by mixing 4 ml (2 ml for 6%) 30% acrylamide/bis solution, 2.5 ml 4× separation gel buffer, 3.5 ml (5.5 ml for 6%) water, 50 ml 10% (w/v) ammonium persulfate solution, and 10 µl TEMED per a 1.5-mm thick gel. Pour the mixture in a glass gel plate, leaving space for stacking gel, and overlay with isobutanol. Polymerization will take about 20 min (see Note 7).
3. Remove the isobutanol and rinse the top of the gel three times with water.
4. Prepare stacking gel solution by mixing 0.5 ml 30% acrylamide/bis solution, 1 ml 4× stacking gel buffer, 2.5 ml water, 30 µl 10% (w/v) ammonium persulfate solution, and 5 µl TEMED per a 1.5-mm thick gel. Pour the mixture to top of the separate gel and insert a 15-well comb. Polymerization will take about 20 min.
5. Prepare running buffer by diluting one part of 10× running buffer with nine parts of water.
6. Set the gel to running chamber and pour the running buffer to the upper and lower chambers. Load 35 µl of each sample in a well and add 3 ml of prestained protein standard.
7. After completing assembly of units, run the gel at 100 V. When dye front is close to bottom of the gel (this will take about 2 h), stop and proceed to next step (see Note 8).

3.4. Detection of Phosphorylated LRRK2 and MBP

1. The amount of LRRK2 and MBP that has been loaded on the gel can be visualized by staining with coomassie brilliant blue. For staining, fix the gel with 10% (v/v) acetic acid and 50% (v/v) methanol for 30 min on low-speed shaker.
2. Prepare coomassie brilliant blue solution as described in user manual. Briefly, mix 55 ml water, 20 ml methanol, 20 ml stainer A, and 5 ml stainer B for two gels. Pour the staining solution to gel and shake the gel for 1–2 h.
3. Decant staining solution when LRRK2 and MBP proteins clearly appear on the gel and rinse the gel with water. Shake the gel and replace water until background is clear.
4. Place the gel between sheet protectors and seal with sealer.
5. Place the sealed gel on the Storage Phosphor Screen Cassette and cover it with Storage Phosphor Screen. Expose gel to Storage Phosphor Screen overnight.

⁷The protein sizes of LRRK2 and MBP are around 280 and 22 kDa, respectively. To resolve these two proteins on a single gel, a 12% SDS-PAGE gel or a 8–16% SDS-PAGE gradient gel must be used (*see* Subheading 3.3).

⁸Because unincorporated radioisotopes are located at the dye front on the gel, be careful that the dye is not run off the gel. Before staining the gel, cut off dye front to avoid high background signals on X-ray films (*see* Subheading 3.3).

6. Read the Storage Phosphor Screen with a Phosphoimager and analyze by the ImageQuant 6.0 software.

3.5. Western Blotting for LRRK2 Expression

1. These instructions are for the Mini-Trans Blot Cell from Bio-Rad Laboratories. Other western blotting equipment may also be used. Prepare 1 L of 1× transfer buffer containing 20% methanol and hold ready a tray that is large enough to submerge transfer cassette. Cut two sheets of Whatman 3 MM paper to the size of the transfer cassette foam pad and a sheet of the nitrocellulose membrane larger than the size of the separating gel.
2. Pour the transfer buffer to the tray and place the transfer cassette with a piece of foam pad and a sheet of Whatman 3 MM paper on one side of the cassette.
3. Disassemble the gel units and remove the stacking gel. After rinsing the gel with water, place it on a sheet of wet Whatman 3 MM paper and lay the wet nitrocellulose membrane on top of the gel. A further sheet of wet Whatman 3 MM paper is laid on the nitrocellulose membrane. Be careful that no bubbles are trapped in the resulting sandwich. The second wet foam pad is laid on the top and the transfer cassette is closed.
4. The cassette is placed in the transfer tank such that the nitro-cellulose membrane is between the gel and the anode. Place a cooling unit in the tank and fill it with transfer buffer. To maintain a low buffer temperature, a refrigerated/circulating water bath can be used. Alternatively, the tank can be placed on ice.
5. Transfer can be accomplished at either 100 V for 2 h or 25 V overnight.
6. Once the transfer is done, disassemble the units and rinse the nitrocellulose membrane with water. Incubate the nitrocellulose membrane with blocking solution for 30 min on low-speed shaker.
7. Discard the blocking solution and then rinse the nitrocellulose membrane with TBST.
8. Horseradish peroxidase (HRP) conjugated antibodies against most of epitope-tags are available. By using these antibodies, you can save incubation times for secondary antibody (see step 11). Dilute the antibody by 1:5,000 in antibody dilution buffer and incubate the nitrocellulose for 1–2 h at room temperature on a low-speed shaker.
9. The antibody is then removed and the membrane is washed four times for 10 min with TBST on low-speed shaker.
10. If you used HRP-conjugated antibodies, skip this step (and step 12). Otherwise, prepare the secondary antibody as a 1:10,000 dilution in antibody dilution buffer and incubate with the nitrocellulose membrane for 30 min at room temperature on low-speed shaker.
11. Discard the secondary antibody and wash the membrane four times for 10 min with TBST on low-speed shaker.

12. Prepare ECL reagent (e.g., Thermo Scientific) by mixing one part of luminol/enhancer solution and one part of stable peroxide solution. Be careful not to contaminate each solution. Place the membrane on sheet protector, blotted with Kim-Wipes, and then add ECL reagent on the membrane. After covering the membrane with the other side of sheet protector, blot the excess ECL reagent with Kim-Wipes.
13. This step is done in a dark room under safe light conditions. The sheet protector containing the membrane is placed in an X-ray film cassette with X-ray film for a suitable exposure time. The film can be developed using a standard X-ray film processor.

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References

1. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging*. 2003; 24:197–211. [PubMed: 12498954]
2. Morris HR. Genetics of Parkinson's disease. *Ann Med*. 2005; 37:86–96. [PubMed: 16026116]
3. Lesage S, Brice A. Parkinson's disease: from monogenic forms to genetic susceptibility factors. *Hum. Mol. Genet*. 2009; 18:R48–59. [PubMed: 19297401]
4. Elbaz A. LRRK2: bridging the gap between sporadic and hereditary Parkinson's disease. *Lancet Neurol*. 2008; 7:562–564. [PubMed: 18539536]
5. Haugarvoll K, Rademakers R, Kachergus JM, Nuytemans K, Ross OA, Gibson JM, Tan EK, 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 PP, Engelborghs S, Pickut B, Uitti RJ, Foroud T, Nichols WC, Hagenah J, Klein C, Samii A, Zabetian CP, Bonifati V, Van Broeckhoven C, Farrer MJ, Wszolek ZK. Lrrk2 R1441C parkinsonism is clinically similar to sporadic Parkinson disease. *Neurology*. 2008; 70:1456–1460. [PubMed: 18337586]
6. Change N, Mercier G, Lucotte G. Genetic screening of the G2019S mutation of the LRRK2 gene in Southwest European, North African, and Sephardic Jewish subjects. *Genet. Test*. 2008; 12:333–339. [PubMed: 18666856]
7. Tan EK, Tan LC, Lim HQ, Li R, Tang M, Yih Y, Pavanni R, Prakash KM, Fook-Chong S, Zhao Y. LRRK2 R1628P increases risk of Parkinson's disease: replication evidence. *Hum. Genet*. 2008; 124:287–288. [PubMed: 18781329]
8. Tomiyama H, Mizuta I, Li Y, Funayama M, Yoshino H, Li L, Murata M, Yamamoto M, Kubo S, Mizuno Y, Toda T, Hattori N. LRRK2 P755L variant in sporadic Parkinson's disease. *J. Hum. Genet*. 2008; 53:1012–1015. [PubMed: 18923807]
9. Floris G, Cannas A, Solla P, Murru MR, Tranquilli S, Corongiu D, Rolesu M, Cuccu S, Sardu C, Marrosu F, Marrosu MG. Genetic analysis for five LRRK2 mutations in a Sardinian parkinsonian population: Importance of G2019S and R1441C mutations in sporadic Parkinson's disease patients. *Parkinsonism Relat. Disord*. 2009; 15:277–280. [PubMed: 18805725]
10. Gorostidi A, Ruiz-Martinez J, Lopez de Munain A, Alzualde A, Marti Masso JF. LRRK2 G2019S and R1441G mutations associated with Parkinson's disease are common in the Basque Country, but relative prevalence is determined by ethnicity. *Neurogenetics*. 2009; 10:157–159. [PubMed: 19020907]
11. Patra B, Parsian AJ, Racette BA, Zhao JH, Perlmutter JS, Parsian A. LRRK2 gene G2019S mutation and SNPs [haplotypes] in subtypes of Parkinson's disease. *Parkinsonism Relat. Disord*. 2009; 15:175–180. [PubMed: 18752982]

12. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, Kachergus J, Hulihan M, Uitti RJ, Calne DB, Stoessl AJ, Pfeiffer RF, Patenge N, Carbajal IC, Vieregge P, Asmus F, Muller-Myhsok B, Dickson DW, Meitinger T, Strom TM, Wszolek ZK, Gasser T. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron*. 2004; 44:601–607. [PubMed: 15541309]
13. Greggio E, Jain S, Kingsbury A, Bandopadhyay R, Lewis P, Kaganovich A, van der Brug MP, Beilina A, Blackinton J, Thomas KJ, Ahmad R, Miller DW, Kesavapany S, Singleton A, Lees A, Harvey RJ, Harvey K, Cookson MR. Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. *Neurobiol. Dis.* 2006; 23:329–341. [PubMed: 16750377]
14. Smith WW, Pei Z, Jiang H, Dawson VL, Dawson TM, Ross CA. Kinase activity of mutant LRRK2 mediates neuronal toxicity. *Nat. Neurosci.* 2006; 9:1231–1233. [PubMed: 16980962]
15. Cookson MR, Dauer W, Dawson T, Fon EA, Guo M, Shen J. The roles of kinases in familial Parkinson's disease. *J. Neurosci.* 2007; 27:11865–11868. [PubMed: 17978026]
16. West AB, Moore DJ, Choi C, Andrabi SA, Li X, Dikeman D, Biskup S, Zhang Z, Lim KL, Dawson VL, Dawson TM. Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity. *Hum. Mol. Genet.* 2007; 16:223–232. [PubMed: 17200152]
17. West AB, Moore DJ, Biskup S, Bugayenko A, Smith WW, Ross CA, Dawson VL, Dawson TM. Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc. Natl. Acad. Sci. USA.* 2005; 102:16842–16847. [PubMed: 16269541]
18. Gloeckner CJ, Kinkl N, Schumacher A, Braun RJ, O'Neill E, Meitinger T, Kolch W, Prokisch H, Ueffing M. The Parkinson disease causing LRRK2 mutation I2020T is associated with increased kinase activity. *Hum. Mol. Genet.* 2006; 15:223–232. [PubMed: 16321986]
19. Covy JP, Giasson BI. Identification of compounds that inhibit the kinase activity of leucine-rich repeat kinase 2. *Biochem. Biophys. Res. Commun.* 2009; 378:473–477. [PubMed: 19027715]