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Regulation of Physiologic Actions of LRRK2: Focus on Autophagy

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Key Words
LRRK2 mutations · Autophagy · Familial and sporadic Parkinson's disease

Abstract
Background: Mutations in LRRK2 are associated with familial and sporadic Parkinson's disease (PD). Subjects with PD caused by LRRK2 mutations show pleiotropic pathology that can involve inclusions containing α-synuclein, tau or neither protein. The mechanisms by which mutations in LRRK2 lead to this pleiotropic pathology remain unknown. ***Objectives:*** To investigate mechanisms by which LRRK2 might cause PD. ***Methods:*** We used systems biology to investigate the transcriptomes from human brains, human blood cells and *Caenorhabditis elegans* expressing wild-type LRRK2. The role of autophagy was tested in lines of *C. elegans* expressing LRRK2, V337M tau or both proteins. Neuronal function was measured by quantifying thrashing. ***Results:*** Genes regulating autophagy were coordinately regulated with LRRK2. *C. elegans* expressing V337M tau showed reduced thrashing, as has been noted previously. Coexpressing mutant LRRK2 (R1441C or G2019S) with V337M tau increased the motor deficits. Treating the lines of *C. elegans* with an mTOR inhibitor that enhances autophagic flux, ridaforolimus, increased the thrashing behavior to the same level as nontransgenic nematodes. ***Conclusion:*** These data support a role for LRRK2 in autophagy, raise the possibility that deficits in autophagy contribute to the pathophysiology of LRRK2, and point to a potential therapeutic approach addressing the pathophysiology of LRRK2 in PD.

Parkinson's disease (PD) is the most common age-related movement disorder. It presents with resting tremor, rigidity, gait disorder, and postural instability. These signs of motor deficiency result from the loss of dopaminergic neurons in the nigrostriatal system. The neuropathological hallmark of PD is the Lewy body, which is composed of aggregated α-synuclein. Mutations in LRRK2 are the most common dominant form of familial PD, and LRRK2-mediated PD exhibits a very high prevalence in specific populations. One striking aspect of LRRK2 pathology is its pleiotropic nature. Cases of LRRK2 typically present with α-synuclein pathology, but can also display tau pathology or no visible inclusion-based pathology [1]. Parkinsonism is commonly exhib-

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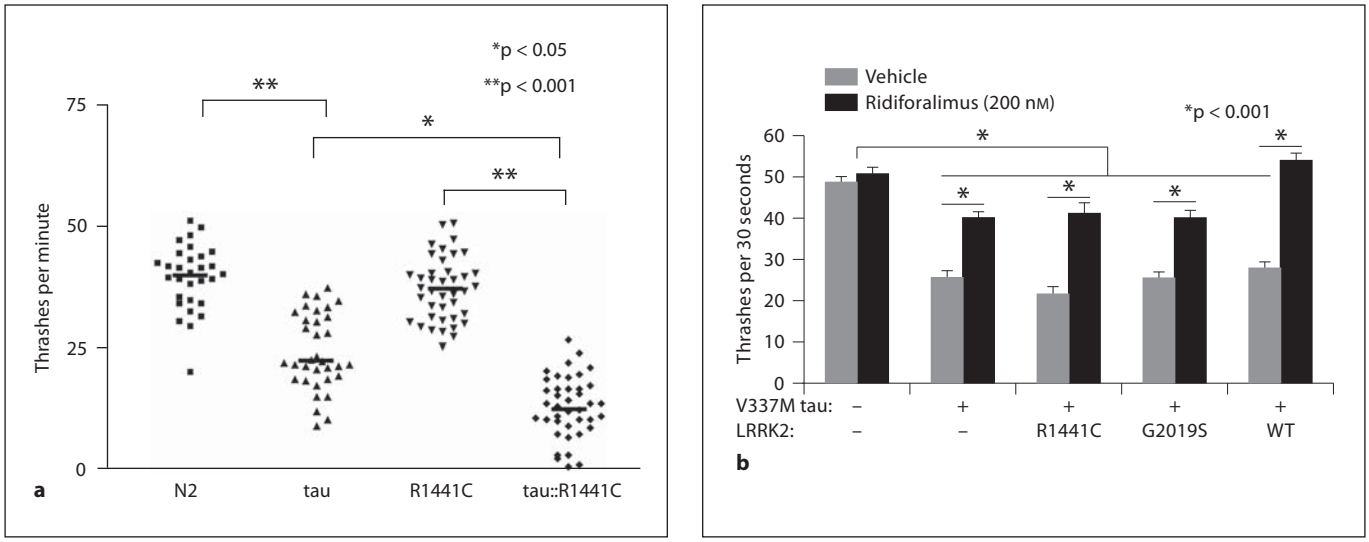

Fig. 1. a R1441C LRRK2 enhances motor deficits caused by expression of tau V337M in *C. elegans*. **b** Ridaforolimus improves motor dysfunction in *C. elegans* expressing V337M tau ± LRRK2 (WT, R1441C or G2019S). WT = Wild type.

ited in subjects with tauopathies. Together, these findings suggest that the pathophysiology of LRRK2-mediated disease might interact with tau in addition to α-synuclein.

The LRRK2 protein is a large ubiquitous cytoplasmic protein with multiple functional domains: kinase, ROC GTPase, Cor, leucine-rich repeat, ankyrin and WD40 domains [1]. The G2019S mutation, found in the kinase domain, is the most prevalent mutation [2–4]. The R1441C mutation, found in the ROC GTPase domain, is conceptually interesting because it appears to decrease GTPase activity [5, 6]. LRRK2 functions as part of a large complex that includes LRRK2 homodimers [7, 8]. Dimerization appears linked to GTPase activity, with dimeric LRRK2 exhibiting more GTPase activity than monomeric [8]. LRRK2 binds many different proteins, including moesin, tubulin, MKK3, 6 and 7, JIP1, 3 and 4, ArhGEF7, DSH, 4E-BP, 14-3-3, HSP90, rab5, rac1, cdc40, parkin and CHIP (reviewed by Greggio and Cookson [1]). LRRK2 appears to participate in several processes including vesicular dynamics, autophagy, neurite extension, mitochondrial function and translational repression mediated by microRNA [9–12]. These data suggest a diverse functional repertoire for LRRK2.

To gain insight into the varied activities of LRRK2, we used systems biology algorithms to develop a functional regulatory network for LRRK2, and transcriptome data from publicly available databases of substantia nigra brain tissue from control and PD subjects, and from blood cells of control and PD subjects. Additionally, we generated arrays from control and transgenic lines of *Caenorhabditis elegans* expressing wild-type LRRK2 treated with and without 25 μM rotenone for 8 h. These conditions are similar to those in our publication describing the LRRK2 *C. elegans* lines.

Once transcriptomes for each condition were obtained, the data were filtered through two different systems biology algorithms. The context likelihood of relatedness algorithm was used to analyze data sets defined by the presence or absence of disease, the human brain and blood cell samples [13]. The algorithm mutual network inference by network identification was used to query the *C. elegans* data, which is state dependent (rotenone treatment) [14]. Genes identified in the resulting regulatory network were then categorized by function. Detailed results are described in a manuscript by Guillily et al. [15]. A wide range of genes showed coordinated regulation with LRRK2. Genes coregulated with LRRK2 included those regulating synaptic transmission, cytoskeletal function, mitochondrial function, protein translation and multiple signaling cascades (e.g. WNT, MAP kinase cascades and NFκB). Genes linked to PD, including parkin, PINK1 and DJ-1, were also coordinately regulated with LRRK2. A subgroup that regulates dopaminergic survival was then identified using RNAi knockdown to identify genes modulating survival of dopamine

neurons after rotenone treatment (250 nM) in *C. elegans* expressing LRRK2 (wild type) and GFP driven by a dopamine transporter promoter. Prior studies show that wild-type LRRK2 enhances survival of dopaminergic neurons under these conditions [12]. Genes linked to autophagy, including other PD genes, showed the most consistent effect on LRRK2 function, improving dopaminergic survival by over 40% as a group.

The strong imprint of genes regulating autophagy on LRRK2 suggests that LRRK2 might impact on disease by modulating autophagy. We began examining the interface between LRRK2 and autophagy by determining whether expressing LRRK2 would affect the response to aggregating proteins. The human LRRK2 (wild type, G2019S or R1441C) line of *C. elegans* was crossed to the human V337M tau line, which exhibits progressive loss of motor function when expressed in *C. elegans* [12, 16]. Although LRRK2 did not modify motor function under basal conditions, coexpressing the two proteins led to a greater loss of motor function than expressing tau or LRRK2 alone (fig. 1a, b). Next, we examined the effects of ridaforolimus (Rid), an mTOR inhibitor that stimulates autophagic flux much like its analog, rapamycin [17]. Rid (200 nM, 3 days) improved movement of *C. elegans* expressing LRRK2 (wild type, G2019S or R1441C) with V337M tau; the wild-type LRRK2 line was particularly responsive, showing movement equal to that of the nontransgenic nematode.

LRRK2 is a large multifunctional protein that interacts with many different proteins. The regulatory network for LRRK2 shows a large array of genes coordinately regulated with LRRK2. This wide range of genes confirms the involvement of LRRK2 in many different cellular functions. The challenge in studying LRRK2 is to distill this wide range of genes down to those most relevant to PD. Our studies suggest genes linked to autophagy exert a particularly strong impact on dopaminergic neuron survival in *C. elegans* lines expressing LRRK2. Prior studies of LRRK2 function in *C. elegans* indicate that disease-linked mutations cause a loss of function. Autophagy plays a critical role in neuronal survival, particularly in the face of stresses such as the accumulation of aggregated proteins. Our study used mutant tau as a source of proteostatic stress, and investigated the effects of Rid, an autophagic inducer. The results demonstrate a striking improvement in motor function following Rid treatment, particularly with wild-type LRRK2. These data support an interaction of LRRK2 with the autophagic system and raise the possibility that mTOR inhibitors, such as Rid, might have utility in therapy of PD.

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