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Mechanisms in dominant parkinsonism; The toxic triangle of LRRK2, α -synuclein and tau

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Abstract

Parkinson's disease (PD) is generally sporadic but a number of genetic diseases have parkinsonism as a clinical feature. Two dominant genes, α -synuclein and leucine rich repeat kinase 2 (LRRK2), are important for understanding inherited and sporadic PD. α -Synuclein is a major component of pathological inclusions termed Lewy bodies found in PD. LRRK2, is found in a significant proportion of PD cases. These two proteins may be linked as most LRRK2 PD cases have α -synuclein positive Lewy bodies. Mutations in both proteins are associated with toxic effects in model systems although mechanisms are unclear. LRRK2 is an intracellular signaling protein possessing both GTPase and kinase activities that may contribute to pathogenicity. A third protein, Tau, is implicated as a risk factor for PD. We discuss the potential relationship between these genes and suggest a model for PD pathogenesis where LRRK2 is upstream of pathogenic effects through alpha-synuclein, tau or both proteins.

Introduction

Understanding human diseases at some level has to involve the root cause, or etiology, of that disease. In some cases this is simple; in an infectious disease a causative agent (virus, bacteria or parasite) can be isolated and used to replicate the disease in a model organism, according to Koch's postulates. But many major diseases have no known cause and are therefore considered sporadic. How might we approach understanding a disease where we cannot identify a single causative agent? Without etiology, much of our work has to be descriptive, our models of the pathogenic mechanisms miss the deepest parts of causation and our treatments are based on symptoms not mechanism.

One way to begin to understand sporadic diseases is to look for inherited diseases with which they share some pathological features. By identifying specific biological pathways in the genetic disease one might then be able to extrapolate back to the mysterious sporadic

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case. Although this may seem to be farfetched, there are examples of where it is starting to reach clinical testing. In Alzheimer's disease the theoretical idea that all cases share a common molecular pathway to neuropathology was named the amyloid cascade hypothesis by Hardy and colleagues¹. This has led to the development of drugs² and immunological approaches³ that are currently in clinical trials.

A major advantage of looking for genetic factors for diseases compared to (e.g.) environmental contributors is that there are defined ways to assign gene variants as pathogenic. For rare variants within families, a gene is causal if it is inherited in either a dominant or recessive manner, and thus shows segregation with the trait of interest. These Mendelian variants often have strong effects on protein function. Gene variants can also be considered pathogenic if they show association with a given disease. This occurs at a population rather than a family level, and is when a given gene variant is at a different frequency in disease cases and controls. The effects of genetic risk factors tend to be subtler, raising or lowering lifetime risk of disease. Although this has traditionally been approached one gene at a time, more recently genome-wide association studies (GWAS) have been used to identify all common genetic risk factors for a given disease and rank them based on the strength of the effect.

Here we will discuss Parkinson's disease (PD), where identification of genetic contributions has revolutionized our thinking about the disease process. We will focus on two genes that cause dominantly inherited disease that may be related to sporadic PD. We will also discuss data from genetic association studies, including GWAS, that supports the idea that there are causal mechanistic relationships between different genetic variants. For a more complete picture of the genetics of PD and parkinsonism, including rare recessive forms that mimic some or all of the PD phenotype, the reader is directed to several recent reviews⁴⁻⁶.

An abbreviated Genetic Neuropathology of Parkinson's disease

PD can be, perhaps simplistically, viewed as a disease of two parts. First, as in all neurodegenerative conditions, there is a loss of neurons in a variety of brain regions. In PD, we often think of a group of neurons in the substantia nigra pars compacta, part of the basal ganglia. Nigral neurons use the neurotransmitter dopamine to signal to the striatum, and are critical for the starting or stopping movement. They are also pigmented, containing a substance neuromelanin that allows them to be seen in sections from the brain *post mortem* without staining. The extent of neuron loss in advanced PD is so severe that the nigra becomes depigmented, and it is thought that substantial numbers of cells are lost by the time symptoms begin. Because these neurons are involved in movement, for most people with PD, movement problems (shaking, slowness of movement, stiffness, unstable posture) are the first symptoms to be noted. This clinical outcome is termed parkinsonism.

The second neuropathological event in PD is deposits of proteins in remaining neurons. Protein deposition is common to many neurodegenerative diseases, and is usually associated with decreased solubility and/or increased aggregation of the protein involved. For example, in Alzheimer's diseases there are aggregates of the A β peptide (a proteolytic fragment of the membrane protein APP) outside of neurons called plaques and aggregated tau protein inside cells that forms tangles. For PD, the characteristic protein deposition is a Lewy body,

predominantly made of a small protein called α -synuclein as well as other protein and lipids. Using α -synuclein as a marker reveals that many brain regions are involved in PD, and has allowed development of a scheme describing the pathology as the disease progresses⁷.

Of the known genes for PD, dominant mutations in the genes for α -synuclein (*SNCA*) and leucine rich repeat kinase 2 (*LRRK2*), stand out due to their potential importance in understanding the relationship between inherited and sporadic PD. Mutations in *LRRK2* cause a parkinsonism syndrome clinically similar to sporadic PD, suggesting that inherited and non-inherited PD might share mechanisms. Interestingly, *LRRK2* mutations account for between 1% and 30% of all PD cases in different populations⁸, again emphasizing how similar *LRRK2* mutations are to typical PD. *LRRK2* codes for a large, complex protein, which has attracted attention as a therapeutic target for PD^{9,10}.

α -Synuclein is important because the protein is the major component of Lewy bodies and related pathology¹¹. Mutations in the *SNCA* gene cause a syndrome that is similar to sporadic PD, although sometimes with earlier onset and a more aggressive course^{12–14}. The amount of α -synuclein protein is important, as shown by multiplication mutations^{15–17} and by association of common genetic variants around *SNCA* with PD^{18,19}. Critically, the majority of *LRRK2* cases that have come to autopsy have α -synuclein positive Lewy bodies in various brain regions²⁰. This potentially links *LRRK2* to α -synuclein pathology and again reinforces the link between genetic diseases and sporadic PD pathology.

Although deposition of the protein tau is associated with Alzheimer's disease, *MAPT* gene mutations cause frontotemporal dementia (FTD) with parkinsonism²¹. Therefore, loss of neurons in the substantia nigra can be driven by tau. Importantly *MAPT* has been nominated as a candidate for gene association with sporadic PD and is a significant risk factor for sporadic PD in a genome-wide association study for PD¹⁹. Unlike α -synuclein, tau is not deposited as a pathological protein in PD, and so the mechanism(s) underlying its contribution to sporadic PD are not clear. Initial data looking at the relationship between the gene variants that increase risk and expression of tau suggests that having more tau is detrimental¹⁹. If confirmed, this would mean that like α -synuclein, the normal human tau protein can contribute to neurodegeneration if there is too much of it.

Collectively, these data identify three suspects that might play pathogenic roles in inherited and sporadic PD. We will next discuss what these gene products normally do in the CNS and how mutations are thought to affect normal function as this can be a first clue to understanding why mutations cause disease.

LRRK2 is an intracellular signaling molecule

LRRK2 is a 2527 amino-acid long protein with a catalytic core region composed of the ROCO protein family²² signature ROC-COR bidomain followed by a kinase domain (Figure 1). The ROC domain is named for Ras of complex proteins as it has some homology to small GTPases including Ras. The COR domain is characteristic of the ROCO protein family and is so named because it is C-terminal of ROC. The kinase domain is generally similar to other Ser/Thr type protein kinases, although it is only close to the *LRRK2* homolog, *LRRK1*, in humans. The catalytic core is flanked N-terminally by a leucine rich

repeat domain and C-terminally by a WD40 repeat domain. In its N-terminal region, LRRK2 displays a large number of unusual repeat sequences termed either LRRK2 or ARM/HEAT repeats^{23–25} (Figure 1). Pathogenic mutations are concentrated in the catalytic center of the protein, including R1441C/G/H in the ROC domain, Y1699C in the COR region and G2019S and I2020T in the kinase domain²⁶.

There is evidence that the different regions of LRRK2 containing PD-associated mutations communicate with each other. LRRK2 self-interacts^{27,28}, exists predominantly in a dimeric conformation^{29–31} and possesses kinase activity in the dimeric state³¹. Intramolecular interactions in the central ROC-COR portion of LRRK2 occur within each monomer chain and collectively contribute to dimer formation. The dimer of LRRK2 may include ROC:ROC interactions^{30,32} but there are stronger ROC:COR^{29,32} and there are COR:COR interactions in more distant homologues of LRRK2 in other species³³. Additionally, both the N- and C-terminal domains of human LRRK2 have been shown to interact³⁰ and a deletion mutant of LRRK2 lacking the central ROC-COR domain retains the ability to dimerize²⁹.

Importantly, R1441, the site of three pathogenic substitutions mediates part of the intramolecular interactions seen in LRRK2³². The equivalent residue to Y1699 in the COR domain of a prokaryotic protein is placed at the ROC:COR molecular interface³³. Local interactions at the ROC:COR interface are disrupted by these mutations²⁹ and thus may subtly influence dimerization. If, as proposed³⁴, dimerization regulates GTPase activity in complex proteins then such interactions may be important in the regulation of normal function of LRRK2 and may provide a link between that function and the pathogenic effects of mutations.

Unfortunately, the normal function of LRRK2 is not yet defined but the presence of both a ROC/GTPase and a kinase domain suggests that it may play a role in intracellular signaling. Generally, GTPases act as molecular switches for other effector proteins and kinases are often outputs for signaling pathways. A widely discussed hypothesis is that the ROC domain in its GTP-bound state stimulates kinase activity (Figure 2). The presence of non-hydrolyzable GTP analogues enhances LRRK2 kinase activity^{35,36}, although the effect is quite modest and incubation with GDP does not completely block it^{36,37}. Pathogenic mutations in the ROC domain disrupt GTPase activity^{38,39} but do not consistently increase kinase activity^{37,40–43}. These data suggest that a simple regulation of kinase by ROC does not explain how all mutations in LRRK2 cause PD.

A limitation of the above data is that there is no currently accepted substrate for LRRK2. Proposed substrates of LRRK2 kinase activity include moesin⁴³, 4EBP⁴⁴ or β -tubulin-2C⁴⁵ but whether these are physiological is unclear. In the case of moesin, LRRK2 is not rate-limiting for phosphorylation in cells⁴⁶ although the phosphorylation state of ezrin-radixin-moesin proteins in neurite filopodia correlates to LRRK2 levels in mouse primary cultures⁴⁷. LRRK2 has been reported to phosphorylate α -synuclein using crude cell extracts as source of kinase⁴⁸, although purified LRRK2 does not phosphorylate α -synuclein⁴⁹. Like many kinases^{50,51}, LRRK2 can phosphorylate itself *in vitro*, which is often used as a measure of activity in the absence of a known substrate. Interestingly, the majority of

autophosphorylation sites in LRRK2 map to the ROC/GTPase domain^{52,53}. This implies that the kinase domain is regulatory to the ROC/GTPase domain, which would then be the major output (Figure 2). If the output signal of LRRK2 is related to the ROC domain, then a search for interaction partners of ROC in its GTP bound form might reveal signaling pathways for LRRK2

Clues for the specificity of LRRK2 function may also come from comparative studies with its only paralog. LRRK1 and LRRK2 have similar domain arrangements and biochemical properties, although LRRK1 is not linked to PD. The major differences between LRRK1 and LRRK2 are at the N-terminal region where LRRK2 has a large number of unique repeats^{23–25}, as well as in the C-terminal region²³. This suggests that LRRK1 has properties that are different from LRRK2, and that both proteins likely impact different signaling pathways. It would be extremely helpful to identify binding partners unique to LRRK2 by examining the N-terminal region.

The above considerations suggest that the normal function of LRRK2 relates to cell signaling, although to which pathways and in which cell types is unclear. Mutations probably change the function of the central GTPase/kinase region of the protein, which then outputs by as yet unidentified interactors to cell signaling.

α -Synuclein and tau have functions specifically relevant to neurons

Most of the above arguments about the function of LRRK2 are influenced by what we know about the domain structure of the protein. The primary sequences of α -synuclein and tau also help us understand function and the effects of mutations.

α -Synuclein is a small protein (Figure 1) with three distinct regions. Towards the N-terminus are a series of repeats that have variations on the sequence KTEGV. In the center of the protein is the NAC region (for non-amyloid component of amyloid plaques, where the fragment was first isolated) that has hydrophobic properties. Finally, at the C-terminus is an acidic tail. The α -synuclein protein is unusual in that it is natively unfolded in solution *in vitro*⁵⁴. This lack of structure leads to a conformational flexibility⁵⁵ that allows it, for example, to bind reversibly to lipid membranes. When bound to lipid vesicles, α -synuclein adopts a helical conformation with part of the helix in the lipid bilayer and the acidic C-terminal tail projecting out into the solvent.

It is thought that lipid binding is important for the normal function of α -synuclein and α -synuclein may have a role in vesicular function. In neurons, α -synuclein is bound loosely to membranes and dissociates rapidly after electrical stimulation⁵⁶. Removing α -synuclein either from neurons leads to a number of subtle changes in vesicles and/or in vesicle release^{57–61}. Additionally, several sets of experiments show that manipulating α -synuclein levels changes lipid composition in the brain^{62–65}. Collectively, these results suggest that the role of α -synuclein in the brain is to influence lipid vesicles, although the exact mechanism(s) involved are not fully identified. Furthermore, as the synucleins are only found in vertebrates, α -synuclein cannot be absolutely required for neuronal function and thus plays a modulatory role in the CNS.

Point mutations in α -synuclein map to the region of KTEGV repeats. Because the normal function of α -synuclein is unclear, it is difficult to say how mutations impact function. Since knockout mice do not develop parkinsonism, and may be resistant to compounds that kill dopamine neurons^{66,67}, and because increased α -synuclein load causes PD, it is not likely that mutations cause disease through loss of normal function. Instead, the protein has some inherent property that can be exaggerated by point mutation or through increase in protein concentration. The most likely, but not definitive, candidate for this is that mutant protein has a higher tendency to aggregate, which will be discussed below in the section on toxic effects of α -synuclein.

In contrast to α -synuclein and LRRK2, the function of tau is better defined as it is a microtubule associated protein⁶⁸. Tau plays important roles in maintaining axonal integrity and morphology. Point mutations in tau cause tauopathies by a combination of loss of the normal binding to microtubules and, as for α -synuclein, an increased tendency to aggregate once dissociated from microtubules⁶⁸. How subtle increases in tau expression associated with sporadic PD would impact the function of axons is unclear but we predict a similarly subtle change in microtubule dynamics.

These data show that the main functions of α -synuclein and tau probably relate to their ability to bind lipids and/or other proteins. Neither protein is absolutely required for neuronal function and both may have modulatory roles. Whether this relates to the subtlety of a disease like PD, with preferential effects in some neurons as patients age, is unclear but it is interesting that both of the biological processes that are affected, synaptic function and microtubule dependent axonal transport, are tasks that neurons specifically have to achieve.

LRRK2 mutations cause neuronal damage

LRRK2 mutations cause Parkinson's disease and cell loss in the substantia nigra has been found in all autopsied LRRK2 positive cases. These considerations suggest that LRRK2 mutations are sufficient to cause dopaminergic cell death in humans. However, individual LRRK2 cases show loss of neurons in the spinal cord associated with mild amyotrophy⁶⁹, cortical pathology associated with dementia^{69,70}, or with psychosis where the anatomic substrate is unclear⁷¹. Therefore, nigral neurons are preferentially vulnerable to mutant LRRK2 although the lesion is not absolutely selective.

However, LRRK2 is present only at low expression levels in the substantia nigra, while structures with extensive connections with the substantia nigra, such as the striatum, show high expression levels of LRRK2^{72–75}. These data suggest that it is possible that there are contributions from other cells. These results are based largely on *in situ* hybridization for mRNA, as well validated antibodies for endogenous LRRK2 are not yet available⁷⁶ and this data may need to be revisited with better reagents.

What, then is the toxic mechanism caused by LRRK2 mutations? Several pieces of data link LRRK2 to cytoskeleton and neurite morphology. LRRK2 has been shown to interact with microtubules⁷⁷ and to phosphorylate β -tubulin 2C⁴⁵. LRRK2 overexpression in transfected SHSY-5Y cells⁷⁸, transfected mouse primary cortical neurons⁷⁹ or transgenic mouse primary neuron cultures^{47,80} leads to reduced neurite outgrowth. While these effects may

partially reflect decreased fitness of cells expressing the mutant protein, reduced neurite length also means that these cells will show reduced connections with other neurons. One potential pathogenic mechanism is that neurons located in regions of relatively high LRRK2 expression (cortex, striatum, hippocampus) may show reduced connections with nigral neurons, leading to reduced viability of these neurons. Therefore, dopaminergic neuronal dysfunction triggered by mutant LRRK2 might include both cell autonomous and non-cell autonomous mechanisms.

These ideas are currently difficult to test since transgenic mice overexpressing mutant forms of LRRK2 appear to lack the main hallmark of PD, namely nigral cell loss^{81,82}, although these models do show impaired functioning of the dopaminergic system and axonal pathology⁸². One way to address these issues is to use techniques leading to region specific overexpression, for instance by testing animals expressing mutant forms of LRRK2 in the striatum compared to animals expressing mutant LRRK2 in the substantia nigra.

From cell culture models the kinase activity of LRRK2 appears to play a role in toxicity^{37,41,83}. These models are based on transient, high level overexpression of LRRK2 and so may not be fully reflective of mechanisms *in vivo*, but they show that kinase dead versions of LRRK2 are relatively innocuous compared to kinase active mutants. There are at least two possible, non mutually exclusive, interpretations of these observations (Figure 2). One is that LRRK2 mutants are kinase activating when expressed in cells and therefore kinase dead versions cannot be hyperactive. A second possibility, if the kinase is a negative regulator of the GTPase domain, kinase dead versions would not switch off the ROC/GTPase domain and thus maintain the protein in a high affinity state (GTP bound) prolonging signaling.

Is the toxic effect of LRRK2 related to normal function? At the time of writing, detailed phenotyping of LRRK2 knockout mice have not been reported, but mention of such animals as part of other studies^{39,72} suggests that the animals don't have a clear parkinsonian phenotype, arguing against a simple dominant negative. In *C. elegans* *lrk-1* (the sole worm homologue of LRRK1 and LRRK2) knockout shows a neuronal polarity phenotype with no overt cell loss⁸⁴ however, the *lrk-1* KO does enhance sensitivity to mitochondrial stressors and G2019S mutant LRRK2 has a loss of protective function⁸⁵. *Drosophila* animals with disrupted dLRRK (the sole fly homolog) show enhanced sensitivity to oxidative stress⁸⁶ and flies overexpressing mutant proteins show the same⁴⁴, again supporting the idea that mutations may mimic loss of function. Clearly, identification of signaling pathways related to mutant LRRK2 will require use of several such model systems (for a more detailed review see⁸⁷).

Another consideration is that the pathogenic function of LRRK2 is unrelated to outputs normally triggered by activity of the wild type protein. If, as discussed above, multiple mutations result in LRRK2 being in a high affinity state then it is possible that the mutant protein gains novel interactors not normally available to wild type LRRK2. Although this is speculative, we propose that screens for GTP-driven outputs should include dominant mutations.

In balance, these data suggest that LRRK2 mutations may work through either gain of detrimental function or loss of a normal putatively protective function or, potentially, though both. Although some work from *in vitro* models suggests that kinase activity is important, these results are very preliminary until robust phenotypes are seen in animal model. Only at such a point can we begin to tease apart how LRRK2 works from where LRRK2 mutations have damaging effects.

α -Synuclein is a toxic protein

As discussed above, one of the key pathological events in PD and related disorders is the deposition of α -synuclein into Lewy bodies. Since this discovery, the question as to whether these protein inclusion bodies are themselves toxic or whether they are the end result of a neuroprotective mechanism has been the subject of debate. Because α -synuclein pathogenic mechanisms have been the subject of numerous reviews, only a brief outline of the main questions will be given here.

As well as likely being important in normal function, the structural flexibility of α -synuclein also underlines a tendency of α -synuclein to aggregate into α -sheet like structures⁸⁸. α -Synuclein is aggregated into fibrillar forms in Lewy bodies⁸⁹, suggesting that the *in vitro* behavior of the protein is related to the pathological forms. However, several lines of evidence suggest that the fibrils themselves are not the toxic species and that intermediates along the aggregation pathway or oligomers are more likely to be detrimental to cells. The A30P and A53T clinical mutant forms of α -synuclein both promote the formation of oligomeric protofibrils, but only A53T robustly promotes the formation of beta-pleated structures *in vitro*^{90–94}. A recent study using structure based design to create variants of α -synuclein that break its propensity to form beta sheets, found that neurotoxicity of α -synuclein could be correlated with the enhanced formation of soluble oligomers⁹⁵. Collectively, these results show that while α -synuclein can be considered a toxic protein the details of the precise toxic species are not fully defined⁹⁶.

Despite a great deal of work being performed in this area, why partially aggregated forms of α -synuclein are toxic to neurons is unclear. Clues from a variety of model systems suggest that the lipid binding capacity of the protein is important in some way, perhaps because α -synuclein can interfere with vesicle transport^{97,98}. Two things about the toxic effects of α -synuclein are clear. First, the expression of α -synuclein is almost entirely neuronal and thus cell-autonomous mechanisms are more likely although some recent results suggest that the protein could transfer between cells⁹⁹. Second, wild type α -synuclein has the potential to be detrimental. This last point is supported by human genetics^{15–19} but also by cell¹⁰⁰ and animal^{101–104} experimental model systems. The difference between mutant and wild type protein is therefore quantitative not qualitative, with α -synuclein toxicity correlating to an excess of an inherent property.

Tau is also a toxic protein. Overexpression of mutated versions associated with frontotemporal dementia and parkinsonism causes neuronal damage in animal models, although formation of tangles *per se* is not always required to kill neurons^{105,106}. Importantly, transient expression of human wild type tau in the substantia nigra causes dopaminergic cell loss and behavioral changes in rats¹⁰⁷. Therefore, there is experimental

evidence to support the idea that an exaggerated normal function of tau can damage the target neurons in PD. We can therefore suggest that a more modest increase of tau protein might sensitize the same neurons to a slower toxic event, driven by other detrimental proteins. In this context, it is interesting that tau is required for the toxic effects of aggregating amyloid protein^{108,109}.

What is the relationship between LRRK2 and α -synuclein? And with Tau?

Most LRRK2 cases have α -synuclein positive Lewy bodies. We recently counted that 14/24 (~60%) of LRRK2 cases reported had Lewy body pathology²⁰. As well as strengthening the link between LRRK2 and sporadic PD, this result implies a relationship between LRRK2 and α -synuclein. Because α -synuclein in Lewy bodies is present in fibrillar structures⁹⁶, we might infer that LRRK2 can promote the aggregation or deposition of α -synuclein, i.e. that LRRK2 is a director and α -synuclein is an actor in PD pathogenesis.

The manner in which LRRK2 may direct α -synuclein aggregation is unclear. Some early work showing the presence of LRRK2 in Lewy bodies suggested that both proteins may interact physically. However, only about a third of these structures were labeled and not all LRRK2 antibodies show this pattern of staining^{75,110}. Therefore, LRRK2 does not seem to be a major protein deposited in PD, although some LRRK2 cases have unusual protein deposits⁶⁹. A recent report⁴⁸ has proposed that LRRK2 controls phosphorylation of α -synuclein at serine 129, although the kinetics are very slow and it is unlikely to be a direct effect⁴⁹. Other kinases, such as G protein coupled receptor kinases (GRK)^{111,112} and polo-like kinase type 2¹¹³ have also been shown to be critical for phosphorylation of α -synuclein *in vivo*. Therefore an important issue will be to determine the relative contribution of LRRK2 to the control of the phosphorylation state of α -synuclein compared to these kinases. Overall, these data support the notion that LRRK2 may direct α -synuclein aggregation through a multi-step process rather than via direct interaction.

What stops us making a simple diagram with one pathway from LRRK2 to α -synuclein are the LRRK2-positive, Lewy body-negative cases. LRRK2 cases without Lewy bodies split approximately evenly between two additional pathologies. Some cases show tau-positive lesions of various types and some¹¹⁴ may have inclusions of TDP43, another protein deposited in the FTD spectrum disorders. This leads to one model that states that LRRK2 can work effectively through α -synuclein *or* tau *or* other aggregating proteins. If we extend this, we can suggest that LRRK2 is 'upstream' of several events involving protein deposition and cell death, a Rosetta stone argument.

However, there is a final group of LRRK2 patients without any specific protein aggregation pathology and 'pure' nigral cell loss^{69,115}. The apparent muteness of these cases speaks to another idea: that LRRK2 can cause cell death without tau or synuclein. Given that the clinical picture of LRRK2 cases is relatively consistent even though pathologies are variable²⁰, this suggests that these two outcomes are independent. What complicates this argument is that we don't definitively know that deposition to form a Lewy body (or a tangle) is a required event in any neurodegenerative disease. To take one example, although mice treated with the toxin MPTP do not form Lewy bodies, α -synuclein knockouts show less MPTP induced neuronal cell death¹¹⁶. This suggests that α -synuclein can contribute to

some toxic events without forming deposits. Furthermore, α -synuclein can promote tau phosphorylation, without full blown tangles¹¹⁷. Therefore LRRK2 may still direct its pathological effects through α -synuclein or tau in the absence of depositions of these proteins.

Figure 3 outlines this framework for thinking about PD, but how could we test such models? It is critical to distinguish necessity from sufficiency for both α -synuclein and tau in mediating the toxic effects of LRRK2. For example, a way to test for the requirement of LRRK2 in tau or α -synuclein toxicity is to test animal models of tau or α -synuclein in animals not expressing LRRK2. Also, to say that LRRK2 is upstream of α -synuclein or tau, we need to show that LRRK2 is not regulated by either. For example, in an animal model of LRRK2 pathogenesis, what happens if we knockout either gene? Such experiments are key to understanding genetic and sporadic forms of PD.

Conclusion

In conclusion, recent advances in α -synuclein and LRRK2 research are refining our framework of thinking about how these genes cause disease. Specifically, LRRK2 is emerging as a molecule containing several elements of an intramolecular signaling cascade and the relationship between various forms of α -synuclein and toxicity is further delineated. Issues that require further clarification are the continued characterization of the intramolecular mechanisms of LRRK2 as well as the identification of the cellular pathways that normal and mutant LRRK2 impact. For α -synuclein, outstanding issues are the further delineation of the precise toxic species as well as the mechanism whereby toxicity is conferred. Finally, the cellular relationship between LRRK2, α -synuclein and tau is a critical question as it may be the basis for developing disease-modifying therapies in both familial and sporadic PD.

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Figure 1.
Domain structures of α synuclein and LRRK2

The primary sequence of the two proteins discussed here is shown to scale with α-synuclein above LRRK2. The box shows a zoomed in view of α-synuclein protein so that the key features can be seen. In both structures, pathogenic mutations are shown in red in the approximate position along the protein. Individual domains are identified below each protein.

Figure 2.

Models of LRRK2 autoregulatory mechanism

Two possible models are shown. On the left is the most widely discussed model that GTP regulates kinase, on the right is an alternative idea, that kinase may be modulatory to GTPase function. In either case, dysregulation is thought to be associated with cell death.

Figure 3.

Modeling the relationship between LRRK2, α -synuclein and tau in PD pathogenic processes.

A proposed relationship between LRRK2, which is upstream and α -synuclein, which is downstream, leading to Parkinson's disease. Note that both α -synuclein and tau are also genetic risk factors for sporadic PD. Arrow thickness correlates with the strength of the process.