MINIREVIEW

Emerging pathways in genetic Parkinson’s disease: tangles, Lewy bodies and LRRK2

Michael J. Devine¹ and Patrick A. Lewis²

¹ Department of Clinical Neuroscience, Imperial College London, UK
² Department of Molecular Neuroscience, Institute of Neurology, University College London, UK

Keywords
α-synuclein; Lewy bodies; LRRK2; MAPT; neurofibrillary tangles; paired helical filament; Parkinson’s disease; RCO protein; SNCA; tau

Correspondence
P. A. Lewis, Department of Molecular Neuroscience, Institute of Neurology, University College London, Queen Square, London WC1N 3BG, UK
Fax: +44 0207 833 1016
Tel: +44 0207 829 8722
E-mail p.lewis@ion.ucl.ac.uk

(Received 7 July 2008, revised 2 September 2008, accepted 24 September 2008)

Introduction

Parkinson’s disease (PD) is characterized by tremor, rigidity and difficulty initiating movement [1]. It is the most common neurodegenerative movement disorder, estimated to affect 100–180 per 100 000, with prevalence rising with age. The economic burden in the UK alone is £6 billion per annum [2]. The pathological hallmark of PD is depigmentation of the substantia nigra pars compacta (SNpc), representing loss of dopaminergic neurons. Abnormal fibrillar cytoplasmic inclusions, Lewy bodies (LBs), are seen in surviving nigrostriatal neurons at autopsy, and these have to be present for a diagnosis of PD to be made [3].

Although the clinical and pathological descriptions of this condition are unambiguous, the cause of cell death remains a mystery. Historically, PD was thought of as a sporadic disorder. Over the last decade, however, familial forms of PD have received widespread attention: 12 loci have been linked to PD, and five responsible genes have been cloned [4]. SNCA, encoding α-synuclein, was the first of these genes to be described. It was later found to be the predominant protein constituent of LBs, suggesting that this protein plays a central part in the pathogenesis of both genetic and sporadic forms of PD [5]. In 1998, mutations in the MAPT gene, encoding the protein tau, were linked to frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), implicating an alternative pathway in the development of parkinsonian conditions [6].

Significant progress was made in 2004 when mutations were first described in leucine-rich repeat kinase 2 (LRRK2) linked to Parkinson’s disease, characterized by variable pathology including either α-synuclein or tau deposition, it has been suggested that LRRK2 functions as an upstream regulator of Parkinson’s disease pathogenesis. This minireview explores this model, in the context of our current understanding of the biochemistry of LRRK2, α-synuclein and tau.
(LRRK2) [7,8]. These mutations are the most common genetic cause of PD with one mutation, G2019S, causing between 2% and 40% (depending on the population) of all PD [9]. LRRK2 PD is phenotypically very similar to sporadic disease, with similar age at onset, clinical presentation, rate of progression, pathological features and response to levodopa, suggesting that there is a shared aetiology. Notably, patients sharing the same mutation can have divergent pathologies [7]. This has led to the suggestion that LRRK2 may link tau and α-synuclein by operating upstream of their respective pathological cascades. Can this model be supported by our current knowledge of these three proteins?

α-Synuclein, Lewy bodies and the synucleinopathies

In 1996 a point mutation, A53T, was described in the α-synuclein gene in a large Greek/Italian PD kindred [10]. Two other missense mutations, A30P and E46K, have since been reported. Subsequently, familial PD cases have been identified which harbour triplications and duplications of α-synuclein [11]. Together, the genetic data imply that quantitative as well as qualitative differences in α-synuclein expression can cause PD.

Function of α-synuclein

The physiological role of α-synuclein is unclear. It is a small 140 amino acid protein, enriched in presynaptic nerve terminals. Natively unfolded, it exhibits environmentally induced conformational plasticity [12]. Mice lacking α-synuclein display disrupted dopaminergic transmission, whereas overexpression of α-synuclein impairs catecholamine release, suggesting that this protein plays a role in modulating neurotransmitter vesicle function, although how this occurs is unknown [13].

Lewy body formation and potential toxicity

Following the identification of mutations in α-synuclein, it was found that amyloid-like fibrils of α-synuclein constitute a major component of LBs in PD and dementia with Lewy bodies (DLB), oligodendroglial inclusions in multiple system atrophy and dystrophic neurites in pantothenate kinase-associated neurodegeneration [14]. These disorders, known as synucleinopathies, are defined by their molecular pathology. LB formation is thought to proceed via fibrillogenic monomers, followed by an oligomeric protofibrillar intermediate. These assemble into fibrils, which are deposited in LBs [15]. A pivotal question is which of these species are toxic to neurons. Several lines of evidence indicate that oligomers may be the perpetrator.

First, pathological studies have shown that LBs are present in 10–15% of individuals over the age of 65 who die without clinical or pathological evidence of neurological illness, with an identical pattern of deposition to that seen in PD or DLB [16]. This suggests that there is dissociation between the presence of LBs and cellular loss, with the latter correlating more closely with PD severity. Furthermore, abundant oligomeric α-synuclein, as well as LBs, deposit in the brains of triplication cases [11].

Second, cytotoxicity in model systems occurs in the absence, or prior to the formation, of aggregated α-synuclein [17]. Lentiviral expression of α-synuclein in rat SNpc results in selective dopaminergic toxicity, but without fibrillar inclusions, and the A30P mutation increases oligomerization of α-synuclein, but not fibril formation [18,19]. This is reinforced by data from a Drosophila model, which suggests that soluble, oligomeric α-synuclein is the toxic species [20]. The mechanism of toxicity is unknown, although there is some evidence that oligomers may cause cytotoxicity by disrupting membranes through the formation of pores [21]. Ironically, given their status as the essential marker for degeneration in PD, aggregation of α-synuclein into LBs may be an adaptive cellular response, similar to aggresome formation, imparting a protective effect.

Regulation of α-synuclein aggregation

In addition to point mutations that alter the propensity of α-synuclein to aggregate, phosphorylation has been shown to play an important role in regulating the aggregation properties of this protein. α-Synuclein has several phosphosite, with phosphorylation at S129 associated with increased aggregation in vitro, toxicity in animal models and with deposition in LBs [22]. This has led to an active search for kinases that can phosphorylate α-synuclein and phosphotases that can strip phosphate groups from the protein. The control of these may play a role in the pathogenesis of PD and offer a potential target for drug treatment.

Tau, tangles and the tauopathies

Just as the synucleinopathies are defined by intracellular aggregates of α-synuclein, the tauopathies, comprising corticobasal degeneration, progressive supranuclear palsy and frontotemporal dementia, are
defined by deposition of the microtubule-binding protein tau. Tau aggregates to form neurofibrillary tangles (NFTs), which are also a component of Alzheimer-type pathology [23]. In 1998, mutations in the tau gene were discovered in FTDP-17, providing a direct link between tau and pathogenesis in these cases [6].

**Function of tau**

Tau is a microtubule-associated protein that is abundant within the central nervous system and exists as six alternatively spliced isoforms [24]. Tau stabilizes microtubules by promoting their polymerization and suppressing their dissociation, and appears to have a stabilizing role during axonal outgrowth [25]. Suppression of tau expression decreases neurite formation, with ectopic expression of tau leading to growth of axon-like structures. Tau isoforms differ by the number of microtubule-binding domains that are present. It is an abundantly phosphorylated protein: phosphorylation has been reported at 30 of a possible 70 serine and threonine residues.

**Tau and pathology**

Similar to amyloid formation by α-synuclein, tau can aggregate to form paired helical filaments (PHFs), which are deposited in NFTs, and evidence suggests that oligomeric tau is the toxic species, rather than NFTs themselves [23]. NFT-bearing neurons can survive for decades and there is no apparent causal relationship between apoptotic morphology and tau deposition. In *Drosophila*, retinal degeneration is observed with tau expression alone, and neuronal death can be seen without NFTs [26]. Suppressing tau expression in a mouse model of FTDP-17 improves memory function and stabilizes neuronal numbers, despite the continued presence of tangles [27].

**Regulation of tau aggregation**

The role of phosphorylation is a critical aspect of the biology of tau. Increased phosphorylation of tau negatively regulates its binding to microtubules and tau is hyperphosphorylated in the PHFs that form NFTs. MAPK activation due to oxidative stress leads to phosphorylation of tau [28]. Following hyperphosphorylation, tau dissociation from microtubules increases the concentration of soluble tau, enhancing its propensity to aggregate [29]. Mutations in tau cause disease by impacting on alternative splicing of exon 10 increasing the ratio of 4R to 3R tau, or point mutations that impair the ability of tau to bind to microtubules.

**Overlap between tau and α-synuclein**

In addition to the pathological parallels between tau and α-synuclein, there is significant overlap at the genetic and clinical levels. Tauopathies often have parkinsonian features, whereas synucleinopathies can present with dementia [30]. Both tau and α-synuclein inclusions have been reported in some disease cases, with LBs and NFTs sometimes present in the same cell. In the Contursi kindred, carrying the A53T SNCA mutation, tau inclusions were observed in some individuals, in addition to LBs [31].

Furthermore, an increase in the overall burden of either α-synuclein or tau can cause disease: triplications of α-synuclein cause PD, whereas the H1 haplotype (which increases transcription of tau) predisposes to tauopathies [32]. *In vitro*, α-synuclein binds tau, can stimulate its phosphorylation and initiate its polymerization [33,34]. Both proteins can synergistically aggregate to form homopolymers.

There is also an interaction in animal models of disease: bigenic mice overexpressing α-synuclein and tau display exacerbated pathologies for both proteins compared with the monogenic equivalents [34]. Finally, a synergistic interaction between tau and α-synuclein has been shown in the development of cognitive impairment in PD patients, where the combination of the MAPT inversion polymorphism and a single nucleotide polymorphism in SNCA doubles the risk of developing cognitive impairment, whereas either alone only marginally increases risk [35].

**LRRK2**

LRRK2 is a large (280 kDa) multidomain protein, with pathogenic mutations causative for PD distributed throughout its length, although there is a degree of clustering within the enzymatic domains [36]. It is a member of the ROCO family of proteins, containing a ROC (Ras of complex proteins)/GTPase, COR (C-terminal of ROC) and a kinase domain, flanked by several protein–protein interaction motifs including a WD40 domain and leucine-rich repeats [37].

**LRRK2 structure and function**

The 3D structure of full-length LRRK2 is unknown, although the crystal structure of the ROC domain suggests that LRRK2 is a dimer, and this is supported by evidence from cellular studies [38,39]. LRRK2 associates with membranous structures including the mitochondrial outer membrane, as well as lysosomal vesicles and punctate structures within the perikarya,
dendrites and axons [40,41]. It is also found in association with Golgi apparatus, plasma membranes and synaptic vesicles.

The physiological function of LRRK2 is unclear. Studies of LRRK2 in rat neurons suggest a role in the regulation of neurite process morphology [42]. Expression of G2019S LRRK2 in SH-SY5Y cells leads to neurite shortening associated with increased autophagic vacuole content, whereas RNA knockdown of autophagy-relevant proteins or interference with the MAPK/ERK signalling pathway reverses this phenotype [43].

LRRK2 interacts with Parkin (mutations in which cause juvenile parkinsonism) in vitro, but not α-synuclein or tau [44]. The ROC domain interacts with β-tubulin, a key component of microtubules, and Moeisin [45,46]. In vivo studies are required to clarify these findings, but these studies suggest interactions with pathways implicated in PD pathogenesis.

**LRRK2 and pathogenesis**

Although patients with mutations in LRRK2 have a uniform PD phenotype, a remarkable feature of these cases is that they display highly variable pathology. Four cases from a clinically uniform R1441C kindred displayed respectively LB pathology typical of PD, a LB distribution similar to DLB, NFTs similar to progressive supranuclear palsy and non-specific nigral degeneration with no observable protein deposition [7]. Cases with the G2019S mutation have variably shown either LB pathology or NFT pathology [47]. This holds true for cases with the Y1699C mutation and a case with the I1371V mutation [48,49]. Four members of the original Japanese kindred, carrying the I2020T mutation, display nigral cell loss without distinct pathology [50].

The G2019S mutation in the kinase domain of LRRK2 has been shown to increase its activity, whereas mutations in the ROC domain disrupt GTPase activity or increase its affinity for GTP [51–53]. These are consistent with a model where the GTPase domain regulates the activity of the kinase domain, with mutations acting to increase the activity of the latter [54]. Not all the experimental data fits with this model – mutations outside the kinase domain do not consistently raise kinase activity by in vitro assay, and a pathogenic mechanism for substitutions in the COR, LRR or WD40 domains altering this has not been elucidated [55].

It is possible that mutations in the COR domain alter the spatial relationship, and thence interaction, between the ROC and kinase domains, and that mutations in the protein/protein interaction domains change binding to substrates or regulatory proteins, but this is speculation in the absence of structural data or physiological substrates.

What is becoming clear is that the enzymatic activities of LRRK2 play a central role in disease, as evidenced by a decrease in the cytotoxicity of mutants if GTP binding or kinase activity are artificially ablated [55,56]. Mirroring the aggregation seen with α-synuclein and tau, there is some evidence that LRRK2 may also aggregate and form inclusions. When LRRK2 is overexpressed in some cells, it can form inclusion bodies that are exacerbated if the protein carries mutations, and LRRK2 has been found in LBs in idiopathic PD [55].

**Links between genetic and sporadic disease**

There are clear pathogenic links between mutations in α-synuclein, tau or LRRK2 and familial PD. However, genetic variability at these loci are also associated with sporadic disease. Genome-wide analysis of sporadic PD has demonstrated linkage to chr17 in the MAPT region [57]. The A0 MAPT allele [58] and H1 haplotype [59,60] are over-represented in the context of sporadic PD. Meanwhile, SNCA is a clear susceptibility gene for PD [61], and a polymorphism in the promoter region of SNCA has been identified as a consistent risk factor for the disease among patients with a variety of ethnic origins. This correlates with increased expression of reporter genes in vitro [62].

Similarly, the LRRK2 G2019S mutation is significantly more common in patients with sporadic PD than in controls [63], with an overall prevalence of 1% in cases of sporadic PD, and 4% in hereditary PD. Furthermore, the common genetic LRRK2 variants G2385R and R1628P both increase the risk of developing sporadic PD in Chinese populations [64,65].

Taken together, these findings suggest that the distinction between sporadic and familial disease is somewhat arbitrary. The extremes of fully penetrant genetic and apparently sporadic PD should be considered opposing ends of a spectrum. Most cases of PD will result from a combination of environmental and genetic factors, underlining the importance of genetic findings in understanding how sporadic disease develops.

As yet there is no evidence for a link between LRRK2 mutations or polymorphisms and tau or synucleinopathies not presenting with pure parkinsonism, for example Alzheimer’s [66], progressive supranuclear palsy [67] or multiple system atrophy [68]. It is not known whether variations in LRRK2 alter the likelihood of developing these diseases – there is no evidence for a direct autosomal dominant causative relationship – or if it plays a
role in directing which pathology prevails, but it would be possible to rationalize such a connection given the unpredictable nature of LRRK2 PD pathology. We anticipate that future genome-wide association studies will bring some clarity.

A pathogenic troika

Individually, mutations in \( \alpha \)-synuclein and tau lead to parkinsonism and dementia characterized by protein deposition in LBs and NFTs respectively, whereas mutations in LRRK2 generate PD coincident with synuclein or tau pathology.

In light of the evidence linking LRRK2 kinase dysfunction to disease, an attractive hypothesis is that tau and \( \alpha \)-synuclein are downstream targets of this activity, linking in to the dysregulation of the phosphorylation state of these proteins. As yet, however, the experimental evidence for this is scant – with only one report of abnormally deposited and phosphorylated tau in a rat neuronal cell model and as yet no reports of cellular dysfunction of \( \alpha \)-synuclein [42]. Based upon the genetic evidence, where \( \alpha \)-synuclein and tau are capable of causing disease independent of mutations in LRRK2, with tau also reacting to deposition of A\( \beta \) in AD, the data suggest that there are separate, yet interlinked, pathways in operation (Fig. 1).

LRRK2: marshalling pleomorphic pathology

One of the most perplexing aspects of LRRK2 pathology is how identical mutations in the same kindred can generate different pathologies. There are several hypotheses, which are not mutually exclusive, for why this might occur.

Impact of genetic variation at other loci

Outside of the context of LRRK2 disease, MAPT haplotypes and SNCA expression levels are known to alter the likelihood of developing corresponding tau or synuclein pathology, so the simplest explanation would be that genetic variability at these loci determine resulting pathology – with the tau and \( \alpha \)-synuclein haplotypes being the obvious candidates. The other possibility is that as yet undetermined gene or genes play a regulatory role, perhaps by providing an interface for cross-talk between the three pathways of LRRK2, tau and synuclein (Fig. 1). To date, the number of LRRK2 cases that have come to autopsy is too small to study for potential genetic modifiers, but this should become tractable with time given the prevalence of mutations, and a compendium of LRRK2 case pathology cross referenced to tau and \( \alpha \)-synuclein haplotype would certainly be of great value.

Impact of environmental factors

Twin studies have suggested that non-genetic factors play a major part in the development of PD. Epidemiological data have suggested several possibly environment-linked factors that affect the likelihood of developing PD, including pesticides, farming and head trauma [69,70].

Fig. 1. The parallel and interlinking pathways between tau, \( \alpha \)-synuclein and LRRK2 neurodegeneration linked to parkinsonism and dementia, showing the potential cross talk between LRRK2 and pathology associated with tau and \( \alpha \)-synuclein dysfunction.
There are also examples from other neurodegenerative diseases. Repetitive head trauma can lead to a dementing state both clinically and pathologically similar to Alzheimer’s disease, termed dementia pugilistica [71]. Subacute sclerosing panencephalitis, which presents with NFT pathology, can arise as a rare late complication of infection with measles [72].

Therefore, different environmental factors might steer LRRK2 either towards tau or α-synuclein pathology. There are two potential pitfalls with examining this hypothesis. First, many of the epidemiological reports on PD do not describe the neuropathology of the cases studied, and so it is currently difficult to build a model linking specific pathologies with specific environmental insults. Second, diseases previously felt to have a robust environmental link have subsequently fallen out of favour: the Parkinson dementia complex of Guam (NFT pathology) is now no longer felt to be due to Cycad exposure [73]. Encephalitis lethargica (another tangle disease) has been argued to be a consequence of influenza, a hypothesis that arose after the contemporaneous outbreaks of encephalitis lethargica and Spanish influenza in the early 20th century, though opinion has since diverged [74].

What is certain is that it will require large numbers of cases of LRRK2 PD with neuropathological correlation and extensive clinical and biographic details to establish any environmental links with certainty.

**LRRK2 as a separate disease entity**

We have discussed evidence suggesting at least some mutations in LRRK2 can dysregulate its kinase activity. Recent evidence in neuronal cell lines suggests that LRRK2 can impact on stress-induced cell death via activation of the ERK pathway in a cell model [75], building upon previous work which found that phosphorylation of proteins on the ERK pathway was reduced in leucocytes carrying the G2019S mutation [76].

A recent study demonstrated a link between LRRK2 activity and phosphorylation of 4E-BP protein in *Drosophila*, again attenuated if LRRK2 is mutated [77]. This protein helps to downregulate protein translation. The authors speculate that in its mutated form, LRRK2 can no longer downregulate formation of those proteins that may, in excess, overwhelm cellular machinery, and start to misfold [78].

These findings each shed light on separate, although not mutually exclusive, mechanisms through which LRRK2 might convey toxicity: through protein misfolding and stress response.

One possible consequence is that the relative balance between a LRRK2 gain of function (via kinase dysregulation and aggregation of proteins) and loss of function (via ERK pathway inactivation or downregulation of protein transcription) determines the pathology that results. The demonstration of phosphorylation and deposition of tau in diseases that are not classically regarded as neurodegenerative (e.g. secondary progressive multiple sclerosis [79]) suggests that tau pathology might be a relatively generic, or default, pathway to neuronal death, with synuclein pathology requiring more stringent provocation. Therefore, one might predict that tau pathology results from LRRK2 loss of function, and synuclein pathology from LRRK2 gain of function. Genetic and environmental modifiers would determine which of these prevails.

Can this account for cases where no specific pathology is seen, for example, in I2020T mutation cases? Perhaps in these circumstances neurons die before typical tau or synuclein pathology can be generated, with a predominance of protofibrils rather than mature aggregates, in which case a further prediction would be that these cases generally have a younger clinical onset of PD and a more rapid clinical course. Current epidemiological data are insufficient to draw conclusions on this.

A bolder alternative is that any observable neuropathology is an epiphenomenon, and that deranged LRRK2 drives neuronal death through a separate pathway, perhaps linked to translation or the ERK pathway. LRRK2 mutations appear to be more specific to PD than either tau or α-synuclein, with the latter two proteins associated with several other neurodegenerative diseases. There is also evidence that neither tau nor α-synuclein accumulation as ordered aggregates is directly correlated with neuronal death. Taken together, LRRK2 may steer pathology without the help of the other members of the troika on the way. This suggests that the correlation between clinical phenotype and genotype is more robust than that between phenotype and neuropathology.

Returning to the therapeutic standpoint, clarification of which of these possibilities is pivotal, because these hypotheses suggest that attenuating dysregulated LRRK2 kinase activity may not be sufficient to limit development of PD, at least in some cases.

**Concluding remarks**

Parkinson’s disease pathogenesis is not a simple, linear pathway, universally triggered by a single event. Instead, akin to cancerous cells developing malignant potential through the accumulation of several carcino-
genic mutations, there may be a requirement for multiple insults to occur before dopamine neuron loss ensues. These can be divided into neuronal stressors (e.g. oxidative) and diminished compensatory responses (e.g. impaired proteasomal pathways). The age-related increase in both PD prevalence, and LRRK2 penetrance, are consistent with this view.

Much remains unknown about the normal and abnormal behaviour of the troika of proteins described. There are three fundamental aspects of LRRK2 in particular that need to be clarified in order to understand its role in PD pathogenesis more completely: (a) the in vivo substrates of LRRK2, so that we can begin to work out which intracellular pathways it maps to; (b) the structure of LRRK2, so we can begin to comprehend how it interacts with these pathways; and (c) the means by which LRRK2 modifies tau and α-synuclein biology specifically. Understanding how the pathogenic mutations in LRRK2 impact upon these three characteristics will aid us in the development of mechanistic therapies for PD.

Acknowledgements

This work was funded by the Brain Research Trust. Pathological images of NFTs, Lewy bodies and neuritic plaques courtesy of Professor Tamas Revesz, Queen Square Brain Bank.

References

14 Jellinger KA (2003) Neuropathological spectrum of synucleinopathies. Mov Disord 18(Suppl. 6), S2–12.


71 Schmidt ML, Zhukareva V, Newell KL, Lee VM & Trojanowski JQ (2001) Tau isoform profile and phos-