A Reassessment of the Lewy Body

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Abstract- Lewy body has been linked to Parkinson's disease for almost a century, but its significance in neurodegenerative diseases is not known. Whether it is toxic, protective, or just a bystander has been a subject of debate. Recent advances in molecular and genetic works suggest Lewy bodies are not essential for the diagnosis and pathogenesis of Parkinson's disease. Furthermore, the discovery of gene mutations in PARK8, an autosomal-dominant late-onset parkinsonism with pleomorphic pathology, suggests the clinical expression of neurodegenerative diseases depends more on the anatomical pathways affected rather than any particular "pathological marker".

Key Words: Lewy bodies, α-Synuclein, Tau, PARK8, LRRK2

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INTRODUCTION

The presence of Lewy bodies in the substantia nigra has long been considered the pathological hallmark of Parkinson's disease[(1)]. These eosinophilic spherical inclusion bodies were first described by Friederich Heinrich Lewy in 1912[(2)], and were later named after him by Tretiakoff[(3)], who documented the presence of Lewy bodies in the substantia nigra[(4)]. With better staining techniques, we now appreciate the diffuse nature of these inclusion bodies, not only in terms of topographical distribution within Parkinson's disease[(5)], but also their presence in diverse conditions such as subacute sclerosing panencephalitis[(6)], Down's syndrome[(7)], Hallervorden-Spatz disease[(8)], multiple system atrophy[(9)], dementia with Lewy bodies[(9)], Lewy body variant of Alzheimer's disease[(10)], and progressive supranuclear palsy[(11)]. Lewy bodies have also been found in the substantia nigra of elderly individuals without neurological disease[(12,13)]. On the other hand, not all patients with Parkinson's disease have Lewy bodies. They are typically absent in autosomal recessive juvenile-onset Parkinson's disease with parkin gene mutations[(14-17)]. How Lewy bodies are formed, and their possible role in the pathogenesis of Parkinson's disease, remain unclear.

What are Lewy bodies?

The classical description of a Lewy body is an intraneuronal, eosinophilic spherical body with a central core and a pale-staining peripheral halo[(18)]. It measures 8 to 30 μm in diameter, and stains pink on regular haematoxylin and eosin preparations. Lewy bodies are found

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in abundance in the surviving neurons of the substantia nigra in Parkinson's disease. They may also be found in the locus ceruleus, dorsal motor nucleus of vagus, nucleus basalis of Meynert, limbic and cortical structures[5]. The cortical Lewy bodies tend to have a diffuse structure without a distinct core and halo[18]. Electron-microscopic examination shows the central core to contain granular material, whereas the peripheral halo consists mainly of filamentous structures, radiating from the centre like the spokes of a wheel[19].

Lewy bodies contain a mixture of lipids, proteins, and neurofilaments[20]. The main constituents are α-synuclein and ubiquitin[21,22]. Lewy bodies without α-synuclein have also been described[23]. In the classical well-formed Lewy body, ubiquitin tends to concentrate within the central core, whereas α-synuclein is located mainly in the periphery[20]. Such a separation of ubiquitin and α-synuclein is not present in the diffuse-type of Lewy body.

Besides α-synuclein and ubiquitin, Lewy bodies also contain ubiquitin-mediated enzymes[24,25], ubiquitinated proteins[24], proteasomes[26], proteasome activators, heat-shock proteins, and other centrosome components such as γ-tubulin and pericentrin[24]. They do not contain synaptophysin, β-synuclein, or γ-synuclein. They have very little or no 20S proteasome α subunits and PA28 activator[27].

What is the function of Lewy bodies?

The suspicion that Lewy bodies might be toxic increased when α-synuclein was found in Lewy bodies[21]. This was despite the fact that previous studies did not show an effect of Lewy bodies on cell structure and function[28,29]. Furthermore, Parkinson's disease may occur in the absence of Lewy bodies[15], as demonstrated in post-mortem studies of autosomal recessive young-onset Parkinson's disease with parkin gene mutations[16,17,30,31].

With improvements in immunohistochemical staining techniques, and advances in transgenic and cell culture studies, interest is now focused on the contents of Lewy bodies, in particular α-synuclein and ubiquitin-proteasome complexes. α-Synuclein is a presynaptic protein whose physiological function in humans is not known. α-Synuclein knockout mice develop normally with no evidence of Lewy bodies or dopaminergic cell loss[32]. However, they had reduced striatal dopamine levels without impairment of locomotive function. They also had abnormal stimulus-dependent release of dopamine, suggesting that under normal circumstances, α-synuclein might play a role as a negative regulator of dopamine release. Other studies showed resistance of MPTP-induced neuronal degeneration in α-synuclein knockout mice[33,34], suggesting that MPTP-induced neuronal toxicity is dependent on α-synuclein. Whether the mutant or wild-type α-synuclein is neurotoxic is not known. Transgenic mice with both mutant and wild-type α-synuclein develop inclusion bodies in the substantia nigra and extrastriatal neurons[35-38]. In particular, the wild-type α-synuclein transgenic mice may develop locomotive impairment[35]. Taken together, these findings suggest that α-synuclein associated toxicity is related to a gain of function rather than a loss of function[39]. The functional gain may be due to an interaction between genetic and environmental factors.

The α-synuclein monomer is normally unfolded, but it can aggregate with other α-synuclein monomers or with other proteins (such as neurofilament proteins) to form Lewy neurites in axons[40] and Lewy bodies in neurons. Cell culture studies have shown that α-synuclein can undergo polymerization to form fibrils and intermediate oligomeric protofibrils[41-43]. One current hypothesis is that protofibrils, and not fibrils, are toxic to the cells[44].

The ubiquitin-proteasome system is important for the processing and degradation of unwanted and possibly cytotoxic proteins[45]. The first step is the activation of ubiquitin by ubiquitin-activating enzyme E1. The high-energy intermediate is then attached covalently to target proteins by other enzymes such as the ubiquitin-conjugating enzyme E2 and the ubiquitin ligase E3 to form a polyubiquitinated protein. The ubiquitinated proteins are then degraded by the 26S proteasome complex, with the release of free and reusable ubiquitin by ubiquitin-recycling enzymes. Lewy bodies contain ubiquitin, ubiquitinated proteins, proteasomes, and other compo-

nents of the ubiquitin-proteasome system[24-26]. This suggests that the formation of Lewy bodies is a cytoprotective mechanism, formed in an attempt to degrade unwanted proteins. *In vitro*, α-synuclein is degraded by the ubiquitin-proteasome pathway[46]. However, recent evidence suggests the ubiquitination of α-synuclein in Lewy bodies might be a pathological event, since unmodified α-synuclein can be degraded by proteasome independent of ubiquitin[47]. The controversy as to whether Lewy bodies are toxic, protective, or just an epiphenomenon, remain unresolved.

The contents of Lewy bodies are similar to those of aggresomes, a microtubule organizing centre that is integral to the regulation of abnormal proteins[48]. Lewy bodies, however, have reduced 20S proteasome α subunits, PA28 activator, and other proteolytic enzymes[27]. These may limit the normal functioning of the ubiquitin-proteasome system[49]. Perhaps Lewy bodies are dysfunctional aggresomes[50], formed as a mechanism to contain the unwanted and potentially toxic proteins, unable to be engaged by the degradation process.

Are Lewy bodies essential for the diagnosis of Parkinson's disease?

Parkinson's disease is a heterogeneous group of disorders with multiple causes, both genetic[51] and environmental[52]. The pathogenic mechanisms may saturate or overwhelm the ubiquitin-proteasome system for handling the unwanted protein load, leading to the formation of Lewy bodies. Toxic intermediate oligomeric protofibrils may form, contributing to the toxicity. Lewy bodies are not necessary for cell death in Parkinson's disease, as in the case of parkin mutation[53]. Mutation in parkin results in loss of ubiquitin ligase E3 function[54], which may be necessary for the formation of Lewy bodies. Postmortem studies of patients with parkin mutations generally do not have Lewy bodies[16,17,30,31] - though there has been one exception[55]. This patient had a compound heterozygous mutation. It is possible that mis-sense mutations of the parkin gene may have some ubiquitin ligase activity, and hence allow formation of Lewy bodies[53]. In another report, a patient with homozygous exon deletion in the parkin gene did not have Lewy bodies, but basophilic α-synuclein inclusion bodies were seen in the neuropils of pedunculopontine nucleus[31]. Parkin knockout mice have high dopamine concentrations in the limbic areas, and altered dopamine oxidative metabolism[56]. This evidence indicates that Lewy bodies are not essential for the pathogenesis and diagnosis of Parkinson's disease.

Furthermore, Lewy bodies have been described in a number of other conditions, such as subacute sclerosing panencephalitis[6], Down's syndrome[7], Hallervorden-Spatz disease[8], multiple system atrophy[9], dementia with Lewy bodies[9], Lewy body variant of Alzheimer's disease[10], and progressive supranuclear palsy[11]. They can even be an incidental finding in normal aging[12,13].

Lewy bodies in Parkinson's disease are not restricted to the substantia nigra, but may be seen outside the nigrostriatal system such as in the locus ceruleus, raphe nucleus, nucleus basalis of Meynert, limbic structures, and neocortex. The pathology of Parkinson's disease seems to begin in the medulla, and ascends in the brainstem to involve the substantia nigra, and eventually the neocortex[5].

Cortical Lewy bodies

The presence of cortical Lewy bodies has been associated with dementia[57]. So called "dementia with Lewy bodies" is the second most common dementia, characterized by the clinical triad of fluctuating cognition, visual hallucination, and parkinsonism[58]. These symptoms are similar to Parkinson's disease with dementia[59,60]. Clinically, it may be difficult to differentiate between the two conditions, if not for the arbitrary "one-year rule": patients with dementia onset within the first year of diagnosis of parkinsonism are diagnosed to have dementia with Lewy bodies, and those with dementia occurring much later are more likely to have Parkinson's disease with dementia[58]. This distinction seems arbitrary. Pathologically, the separation is less distinct. Both conditions have Lewy bodies in the brainstem, limbic, and cortical structures[61-64]. Subtle differences may exist. For example, Harding et al.[65] reported more Lewy bodies in the inferotemporal cortex of patients with dementia with Lewy bodies. In Parkinson's disease with dementia, the

Lewy body load is higher in the frontal cortex. Alzheimer's pathology such as neurofibrillary tangles and senile plaques are scattered over the hippocampus, entorhinal cortex, and neocortex in both cases[61,66]. In view of the similarity, it has been suggested that Parkinson's disease with and without dementia, and dementia with Lewy bodies, may represent a spectrum of the same pathological condition[63,64]. It is interesting to note that back in 1923, Lewy had already described the diffuse and cortical distribution of Lewy bodies, and had never suggested making them the pathological hallmark of Parkinson's disease[4].

Is dementia in Parkinson's disease related to Lewy bodies or Alzheimer's pathology? The presence of Lewy bodies in the cerebral cortex has been linked to the development of dementia[57,67,68]. Some authors reported a correlation between cognitive deficits in Parkinson's disease and the Lewy body load in areas such as the entorhinal cortex, anterior cingulate cortex and frontal cortex[69,70]. Churchyard and Lees[71] reported a significant correlation only with the density of Lewy neurites in the hippocampus. Others, however, did not find any association between clinical symptoms and Lewy body densities[61]. Lewy bodies may also be present in the limbic structures and neocortex of Parkinson's disease without dementia[63]. The "Alzheimer's pathology" in Parkinson's disease and dementia with Lewy bodies is usually milder compared to Alzheimer's disease. Alzheimer's pathology up to Braak stage III may be clinically asymptomatic. However, it has been suggested that the additional load of neurofibrillary tangles and senile plaques on a brain laden with Lewy bodies in the limbic system may be additive[72].

The presence of tau and α-synuclein together

Tau is a microtubule-associated protein that is present in neuronal and glial inclusions such as neurofibrillary tangles, Pick's bodies, astrocytic plaques, and coiled bodies[73]. It is found in neurodegenerative diseases such as Alzheimer's disease, Pick's disease, frontotemporal dementia with parkinsonism, progressive supranuclear palsy, and corticobasal ganglia degeneration[74]. These conditions are often grouped together as taupathies. Likewise, Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy are often grouped together as synucleinopathies[9]. This division, again, is arbitrary. Both tau and α-synuclein have been reported in Alzheimer's disease, Parkinson's disease, dementia with Lewy bodies, and progressive supranuclear palsy[11,75]. The two proteins may co-locate within the same neuron in these conditions[11,75]. Epitope-mapping studies have shown similar α-synuclein staining patterns in Lewy bodies of different neurodegenerative diseases[76]. Using double-immunofluorescence staining techniques, Duda et al.[77] were able to show the co-existence of tau and α-synuclein within the same inclusion body in familial Parkinson's disease with α-synuclein gene mutation. Similarly, Ishizawa et al.[78] have shown tau in Lewy bodies of sporadic Parkinson's disease, especially in the locus ceruleus and nucleus basalis of Meynert. Therefore "pathological markers" are not unique to any particular disease, and may co-exist in different neurodegenerative conditions.

Pleomorphism with one etiology

The clinical expressions and pathological changes of neurodegenerative diseases may vary among individuals, even though they may share a common etiology[79]. In the two large families with autosomal-dominant late-onset parkinsonism linked to PARK8 loci on chromosome 12[80-82], the affected members all showed classical parkinsonian features with good response to levodopa therapy. In addition, other features were documented such as dementia, amyotrophy, and supranuclear gaze palsy. Postmortem studies were available in six cases, all without clinical features of dementia. Of these, only one showed classical Lewy bodies in the brainstem. One had diffuse Lewy body disease, another had tau inclusions similar but not diagnostic of progressive supranuclear palsy. The remaining three cases had non-specific nigral cell loss with ubiquitin-positive neuronal inclusions. One of them had mild to moderate "Alzheimer's pathology", the other had mild motor neuron disease. The gene mutations in PARK8 have recently been discovered, and were found to encode for a large, multifunctional protein known as LRRK2 (leucin-rich repeat kinase 2)[79].

LRRK2 belongs to the ROCO protein family, and contains multiple domains that play important roles in protein-protein interactions and regulation of cellular processes[83]. In particular, the kinase activity may be crucial for the phosphorylation of tau and α-synuclein. Therefore a single genetic etiology may give rise to different neurodegenerative conditions such as parkinsonism, dementia, and amyotrophy. Neurodegenerative overlap syndrome has previously been reported[84]. Given time, a "new" neurodegenerative disease may appear. The clinical and pathological expressions may depend on interactions between environmental influences and the common genetic defect[85].

CONCLUSION

We have presented evidence indicating that Lewy bodies are not the "hallmark" of Parkinson's disease. Furthermore current observations show that the pathological expression of one neurodegenerative etiology can vary markedly from one individual to another. In an attempt to integrate the implications of this finding, we suggest that neurodegenerative mechanisms may be set in motion by a variety of causes, and ultimately converge on a final process of neuronal death. The clinical expression of neurodegeneration will be expressed by the anatomical pathways primarily affected rather than any particular pathological marker. There is even evidence to suggest that with the passage of time, there is convergence of the anatomical pathways attacked by neurodegenerative disorder. If a patient presenting with Alzheimer's disease lives long enough, the features of Parkinson's disease will emerge, and vice versa[84].

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