Intraneuronal filamentous tau protein and α-synuclein deposits in neurodegenerative diseases

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Alzheimer’s disease and Parkinson’s disease are the most common neurodegenerative disorders of the human brain. They are characterized by the presence of ordered filamentous assemblies which gradually develop in a small number of nerve cell types. In Alzheimer’s disease, vulnerable nerve cells develop neurofibrillary tangles, neurofilament threads and abnormal neurites, whereas in Parkinson’s disease they develop Lewy bodies and Lewy neurites (reviewed in [1,2]). Alzheimer’s disease is characterized by the additional presence of extracellular deposits in the form of amyloid plaques. Over recent years, it has become clear that the intraneuronal filamentous deposits of Alzheimer’s disease and Parkinson’s disease are composed of tau protein and α-synuclein respectively.

Abbreviations used: NFT, neurofibrillary tangle; PHF, paired helical filament; SF, straight filament.
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A diagnosis of Alzheimer's disease is made when deposits are also the defining neuropathological hallmark of a number of other primary dementing disorders, such as Pick's disease and the emerging group of familial frontotemporal dementias with Parkinsonism that are linked genetically to a region on the long arm of chromosome 17 [4]. Filamentous α-synuclein deposits define dementia with Lewy bodies, a common late-life dementia that exists in a pure form or overlaps with the neuropathological changes of Alzheimer's disease [3,5].

The most common neurodegenerative disorders thus share the deposition within some nerve cells of ordered filamentous assemblies. This assembly into insoluble filaments is abnormal, since these proteins normally exist in a soluble, non-filamentous form. It is likely that nerve cells die as the direct result of the presence of these filaments. This is supported by the finding that in some inherited cases of Parkinson's disease the protein that is mutated is a major component of the filamentous lesions, indicating a direct link between a genetic lesion and the formation of intracytoplasmic filamentous deposits [6]. The same is true of some familial forms of frontotemporal dementia [7].

**Tau protein in Alzheimer's disease**

A diagnosis of Alzheimer's disease is made when a patient exhibits clinical evidence of progressive dementia and when a post-mortem examination of the brain reveals abundant extracellular neuritic plaques and intracellular neurofibrillary lesions. Although these deposits have been known ever since Alois Alzheimer described them in 1907, it is only during the past 14 years that their chemical composition has been elucidated. Extracellular plaques consist of β-amyloid protein Aβ, which is a fragment of the larger amyloid precursor protein [8,9]. Genetic evidence has shown that amyloid precursor protein pathology plays an important role in the aetiology and pathogenesis of at least a proportion of Alzheimer's disease cases [10].

Abundant amyloid deposits can be present in cognitively normal individuals and it is the presence of intracellular neurofibrillary lesions that correlates better with the presence of dementia [11,12]. Moreover, some dementing disorders have only a neurofibrillary pathology. Neurofibrillary lesions form within nerve cells of cerebral cortex, hippocampal formation and some subcortical nuclei. The nerve cells eventually degenerate and it appears likely that they die because they contain neurofibrillary lesions. These lesions are found in nerve cell bodies and apical dendrites as neurofibrillary tangles (NFTs), in distal dendrites as neuropil threads and in the abnormal neurites which are often, but not always, associated with amyloid plaques.

Ultrastructurally, these lesions consist of paired helical filaments (PHFs) and the related straight filaments (SFs). About 95% of filaments are in the form of PHFs, with a diameter of 20 nm and a periodicity of 80 nm. The remainder consists of SFs. Both PHFs and SFs are made of microtubule-associated protein tau, in a hyperphosphorylated state [1].

Tau is a microtubule-associated protein that is involved in microtubule assembly and stabilization. In adult human brain six tau isoforms are expressed which are produced by alternative mRNA splicing from a single gene located on the long arm of chromosome 17 [13]. They differ by the presence of three or four tandem repeats located in the C-terminal region, in conjunction with 0, 29 or 58 amino acid inserts located in the N-terminal region. There is also a larger tau isoform, with an additional 254 amino acid insert in the N-terminal region, which is mainly expressed in the peripheral nervous system [14]. The repeat regions of tau and sequences flanking the repeats constitute the microtubule-binding domains, while the functions of the amino-terminal regions remain unclear [15].

Tau is a phosphoprotein and its mobility on SDS PAGE is affected by phosphorylation. Tau from PHFs is hyperphosphorylated and abnormally phosphorylated relative to tau from normal adult brain [16]. Thus, PHF-tau runs as three major bands of 60, 64 and 68 kDa and a minor band of 72 kDa (Figure 1) [17,18]. Upon dephosphorylation, six tau bands are seen which align with the six recombinant human brain tau isoforms [19]. Several approaches have helped to delineate which tau isoforms make up each PHF-tau band [20–22]. The shortest and longest tau isoforms make up the 60 kDa and 72 kDa bands, respectively. Each of the 64 kDa and 68 kDa bands consists of two tau isoforms, one with three repeats and one with four repeats. PHF-tau from Alzheimer's disease brain thus contains all six tau isoforms, each in a hyperphosphorylated state [20–22].

Hyperphosphorylation and abnormal phosphorylation are major biochemical characteristics of PHF-tau. They are early events in the
Figure 1
Schematic representation of tau bands from filamentous assemblies of different tauopathies

Type I includes Alzheimer’s disease and a number of other dementing disorders; it is characterized by tau bands of 60, 64, 68 and 72 kDa which consist of all six brain tau isoforms. Type II includes Pick’s disease; it is characterized by two major tau bands of 64 and 68 kDa and a minor band of 68 kDa which consist of three tau isoforms, each with three microtubule-binding repeats. Type III includes progressive supranuclear palsy, corticobasal degeneration and familial multiple system tauopathy with presenile dementia; it is characterized by two major bands of 64 and 68 kDa and a minor band of 72 kDa which consist of three tau isoforms, each with four microtubule-binding repeats.

- Ageing
- Alzheimer’s disease
- Down’s syndrome
- Guam Parkinsonism/dementia complex
- Familial presenile dementia with tangles
- Niemann-Pick disease type C
- GSS with tangles
- Progressive supranuclear palsy
- Corticobasal degeneration
- Familial multiple system tauopathy with presenile dementia

The development of the neurofibrillary lesions and, as a result, tau is unable to bind to microtubules [23, 24]. A number of protein kinases and protein phosphatases has been implicated in the abnormal phosphorylation of tau, based largely on in vitro studies of tau phosphorylation. Recent additions to this growing list include a number of stress-activated protein kinases, chiefly stress-activated protein kinase-3 and stress-activated protein kinase-4 [25]. Relatively little is known about which protein kinases phosphorylate tau in brain. This requires specific protein kinase inhibitors or inactivation of individual protein kinase genes. The use of lithium chloride as a specific inhibitor of glycogen synthase kinase-3 and stress-activated protein kinase-4 has provided compelling evidence that this protein kinase is involved in the phosphorylation of tau in normal brain [26, 27].

Whether hyperphosphorylation and abnormal phosphorylation of tau are sufficient for PHF formation is unclear. Phosphorylated recombinant tau has consistently failed to assemble into PHF-like filaments in experiments in vitro. In contrast, incubation of recombinant tau with sulphated glycosaminoglycans, such as heparin or heparan sulphate, results in bulk assembly of tau into Alzheimer-like filaments (Figure 2) [28-30]. Tau isoforms with three repeats assemble into twisted paired helical-like filaments, whereas tau isoforms with four repeats assemble into straight filaments. By immunoelectron microscopy, the paired helical filaments can be decorated by antibodies directed against the N- and C-termini of tau, but not by an antibody directed against the microtubule-binding repeat region [28]. These results, which indicate that in the filaments the repeat region of tau is inaccessible to the antibody, are identical to those previously obtained with PHFs from Alzheimer’s disease brain [19]. They establish that the microtubule-binding repeat region of tau is essential for sulphated glycosaminoglycan-induced filament formation. Three microtubule-binding repeats of tau are believed to form the core of the PHF from Alzheimer’s disease brain [31], supporting the evidence for a similar organization of the two types of filament. The dimensions of tau filaments formed in the presence of sulphated glycosaminoglycans are similar to those extracted from Alzheimer’s disease brain, with a diameter of approximately 20 nm for twisted and 15 nm for straight filaments, with a crossing-over spacing of approximately 80 nm for paired helical-like filaments, although their twist is in general less regular than in Alzheimer’s filaments.

Sulphated glycosaminoglycans also stimulate phosphorylation of tau by a number of protein kinases, prevent the binding of tau to taxol-stabilized microtubules and disassemble microtubules assembled from tau and tubulin [28, 30]. Moreover, heparan sulphate has been detected in nerve cells in the early stages of neurofibrillary degeneration [28, 32]. Sulphated glycosaminoglycans stimulate tau phosphorylation at lower concentrations than those required for filament formation. The pathological presence of heparan sulphate within the cytoplasm of some nerve cells, perhaps as a result of leakage from membrane-bound compartments, might first lead to increased phosphorylation of tau, resulting in its inability to bind to microtubules. At higher heparan sulphate concentrations tau could then assemble into PHFs and SFs. Formation of tau filaments is also observed after incubation of recombinant tau with RNA [30, 33] which has been shown to be sequestered in the neurofibrillary lesions of Alzheimer’s disease [34]. Whether the presence of RNA is an early event remains to be determined. Sulphated glycosaminoglycans and RNA share a repeat sugar backbone and negative charges in the form of sulphates or phosphates. Tau protein is thought to be an extended molecule with little secondary
Figure 2

Heparin- and heparan sulphate-induced filament assembly of recombinant htau37 (381 amino acid isoform of human tau)

(A) Low-power view of filaments formed after incubation of htau37 with heparin. (b) High-power view of heparin-induced twisted tau filaments. (C) htau37 incubated with heparan sulphate. (D) htau37 incubated with heparan sulphate + 10 μM ZnCl₂. Note the presence of paired helical-like filaments in A–D and of additional thin, wavy half-twisted filaments in C. Scale bar (in D): A, 450 nm; B–D, 100 nm (from [30]).
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structure which becomes partially structured upon binding to microtubules. Binding of sulphated glycosaminoglycans or RNA to tau may induce or stabilize a conformation of tau that brings the microtubule-binding repeats of individual molecules into close proximity, creating sites which favour filament formation.

Other tauopathies

Anti-tau antibodies also identify tau pathology in a number of neurodegenerative disorders other than Alzheimer’s disease. Unlike Alzheimer’s disease, there are no Aβ amyloid deposits in many of these diseases; however, like Alzheimer’s disease, the tau lesions stain with available phosphorylation-dependent and phosphorylation-independent anti-tau antibodies. One exception is antibody 12E8 which recognizes tau protein phosphorylated at Ser-262 and/or Ser-356: it fails to stain the tau pathology of Pick’s disease and argyrophilic grain dementia [35,36]. The tau pathology of Alzheimer’s disease is almost entirely confined to nerve cells. This contrasts with tauopathies, such as corticobasal degeneration, progressive supranuclear palsy and familial multiple system tauopathy with presenile dementia, where both nerve cells and glial cells are affected [4]. In corticobasal degeneration and progressive supranuclear palsy, both astrocytes and oligodendrocytes contain tau deposits, whereas in familial multiple system tauopathy with presenile dementia, oligodendrocytes are mainly affected. All tauopathies share a filamentous tau pathology made of hyperphosphorylated tau protein, but are otherwise distinguished in that different nerve cell types and brain regions are affected. Morphologies of tau filaments and their biochemical characteristics also differ between some of these diseases.

Filaments identical to those seen in Alzheimer’s disease are found in Down’s syndrome, dementia with tangles only, familial presenile dementia with tangles, Parkinsonism–dementia complex of Guam, prion diseases with tangles and Niemann–Pick disease type C [4]. Biochemically, the pattern of tau bands is identical to that of Alzheimer’s disease. Straight filaments of slightly differing morphologies are found in Pick’s disease and progressive supranuclear palsy. Filaments in familial multiple system tauopathy with presenile dementia are twisted, with an irregular periodicity of 90–130 nm [21]. They closely resemble tau filaments in corticobasal degeneration [37]. Biochemically, pathological tau from Pick’s disease defines a second type of tau pathology, where the abnormal tau consists of two major bands of 60 and 64 kDa and a minor band of 68 kDa (Figure 1) [38]. The use of isoform-specific anti-tau antibodies, in conjunction with two-dimensional gel electrophoresis, has shown that these bands contain only tau isoforms with three microtubule-binding repeats [39]. Pathological tau from progressive supranuclear palsy, corticobasal degeneration and multiple system tauopathy with presenile dementia runs as two major bands of 64 kDa and 68 kDa and a minor band of 72 kDa, defining a third type of tau pathology (Figure 1) [21,37,40]. It differs from Alzheimer’s disease by the absence of the 60 kDa band. The tau isoform composition has been investigated in familial multiple system tauopathy with presenile dementia and shown to consist only of three tau isoforms, each with four microtubule-binding repeats [21].

Most tauopathies are sporadic diseases, but some are familial and inherited in an autosomal-dominant manner. Interestingly, a number of these tauopathies are familial frontotemporal dementias with Parkinsonism which are linked to chromosome 17q21–22, the same region that contains the tau gene [41]. Intriguingly, in one family with frontotemporal dementia linked to chromosome 17 (Seattle family A), a valine to methionine change is present at residue 337 in tau (in the numbering of the 441 amino acid human tau isoform) [42]. This change segregates with the disease and has not been found in a control population. Neuropathologically, Seattle family A (also known as familial presenile dementia with tangles) is characterized by a neuropathological pathology that is indistinguishable from the tau pathology of Alzheimer’s disease in its ultrastructural and biochemical characteristics [43]. In contrast with Alzheimer’s disease, Aβ amyloid deposits are not present. Together with our discovery of an intronic mutation in the tau gene in familial multiple system tauopathy with presenile dementia [7], this marks the beginnings of the genetics of tau.

α-Synuclein in Lewy body disorders

The Lewy body constitutes the second most common nerve cell pathology, after the neurofibrillary lesions of Alzheimer’s disease. It is the defining neuropathological feature of Parkinson’s disease and dementia with Lewy bodies [2]. Clinically, Parkinson’s disease is a movement...
disorder that is characterized by tremor, rigidity and bradykinesia. Neuropathologically, it is defined by nerve cell loss in the substantia nigra and the presence of Lewy bodies and Lewy neurites. In many cases, Lewy bodies are also found in the dorsal motor nucleus of the vagus, the nucleus basalis of Meynert, the locus coeruleus, the raphe nuclei, the midbrain Edinger–Westphal nucleus, the cerebral cortex, the olfactory bulb, and some autonomic ganglia.

Besides the substantia nigra, nerve cell loss is also found in the dorsal motor nucleus of the vagus, the locus coeruleus and the nucleus basalis of Meynert. Ultrastructurally, Lewy bodies and Lewy neurites consist of abnormal filamentous material [2]. Lewy bodies and Lewy neurites also constitute the defining neuropathological characteristics of dementia with Lewy bodies, a common late-life dementia that exists in a pure form or overlaps with the neuropathological characteristics of Alzheimer’s disease.

Unlike Parkinson’s disease, dementia with Lewy bodies is characterized by large numbers of Lewy bodies in cortical brain areas. However, Lewy bodies and Lewy neurites are also present in substantia nigra in dementia with Lewy bodies, whereas hippocampal Lewy neurites are found in a proportion of individuals with Parkinson’s disease with severe cognitive impairment. Disorders with Lewy bodies and Lewy neurites thus present as a clinical and neuropathological spectrum. Classical Parkinson’s disease with minor cognitive impairment and minimal cortical pathology is at one end of the spectrum, whereas severe dementia, with or without antecedent Parkinsonism, but with a severe cortical and hippocampal Lewy body and Lewy neurite pathology, is at the other end of the spectrum. Despite the fact that the Lewy body was first described in 1912, its biochemical composition has remained unknown.

Over the past year, the discovery of point mutations in the α-synuclein gene as a rare cause of familial Parkinson’s disease [6,44] has led us to examine the presence of α-synuclein in Lewy bodies and Lewy neurites in idiopathic Parkinson’s disease and in dementia with Lewy bodies. Human α-synuclein is 140 amino acids in length and is abundantly expressed in brain, where it is located in presynaptic nerve terminals, with very little staining of nerve cell bodies and dendrites [45–48]. Two related proteins, called β-synuclein and γ-synuclein (or BCSG1 protein), have been described [45–48]. The amino-terminal half of each synuclein is taken up by imperfect amino acid repeats, with the consensus sequence Lys-Thr-Lys-Glu-Gly-Val. The repeats are followed by a hydrophobic middle region and a negatively charged C-terminal region. To stain Lewy bodies and Lewy neurites, we used two antibodies raised against synthetic peptides corresponding to residues 11–34 (PER1) and 116–131 (PER2) of human α-synuclein [3,5]. On immunoblots, these antibodies recognize α-synuclein, but not β- or γ-synuclein. PER1 and PER2 gave strong staining of Lewy bodies and Lewy neurites in substantia nigra from Parkinson’s disease brain (Figure 3). Both the core and the halo of the Lewy body were strongly immunoreactive for α-synuclein. Similarly, in dementia with Lewy bodies, the anti-α-synuclein antibodies PER1 and PER2 stained both brainstem and cortical Lewy bodies, as well as numerous Lewy neurites. In contrast, antibodies specific for β- or γ-synuclein failed to stain Lewy bodies and Lewy neurites. Thus, of the three known brain synucleins, only α-synuclein is found in Lewy bodies and Lewy neurites.

Double-staining for α-synuclein and ubiquitin showed labelling of most Lewy bodies with both antibodies [5]. Ubiquitin antibodies frequently stained only the halo of brainstem-type Lewy bodies, whereas α-synuclein antibodies stained both the halo and the core. Some Lewy bodies were immunoreactive for α-synuclein, but not for ubiquitin. This was also true of Lewy neurites, where the α-synuclein-positive neurites outnumbered those immunoreactive for ubiquitin or neurofilaments. Staining for α-synuclein will probably replace staining for ubiquitin as the preferred means of identifying Lewy bodies and Lewy neurites.

These findings show that α-synuclein is a component of the Lewy body and Lewy neurite. In conjunction with mutations in α-synuclein as a rare cause of Parkinson’s disease, they suggest but do not prove that α-synuclein is a major component of the abnormal filaments that make up Lewy bodies and Lewy neurites. We investigated this directly by immuno-electron microscopy of sarcosyl-insoluble filaments extracted from cingulate cortex of patients with dementia with Lewy bodies [5]. Filaments had a diameter of 5–10 nm, in keeping with previously reported dimensions of filaments in Lewy bodies and Lewy neurites, based on heavy-metal-stained tissue sections and isolated Lewy bodies. Anti-
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serum PER4, which recognizes the C terminus of \( \alpha \)-synuclein, labelled filaments along their entire lengths, indicating that they contain \( \alpha \)-synuclein as a major component. The labelled structures had various morphologies, including a 5 nm straight filament and both straight and twisted 10 nm filaments; the 10 nm filaments were more numerous. These appearances would be consistent with a model in which the \( \alpha \)-synuclein molecules assembled to form a 5 nm protofilament, two of which could associate to produce a variably twisted filament. Some 10 nm filaments were unravelled, revealing slender extensions at one or both ends. These various morphologies suggest that \( \alpha \)-synuclein molecules, which are believed to be extended and relatively unstructured, may run parallel with the filament axis. This differs from the packing of tau protein in filaments from Alzheimer’s disease and other tauopathies, where individual tau molecules are believed to run mainly perpendicular to the filament axis [49]. Antibody PER1, which was raised against residues 11–34 of \( \alpha \)-synuclein, only ever labelled one filament end. This suggests firstly that the PER1 epitope is buried in the body of the filament and exposed only at the end, and secondly that the filaments are polar structures. Although PER1 labelled only one end of each \( \alpha \)-synuclein filament, it stained the Lewy body pathology very strongly. This suggests the presence of a pool of unassembled \( \alpha \)-synuclein in Lewy bodies and Lewy neurites. In Alzheimer’s...

Figure 3

Substantia nigra from patients with Parkinson’s disease immunostained for \( \alpha \)-synuclein (antibody PER2)

(A) Two pigmented nerve cells, each containing an \( \alpha \)-synuclein-positive Lewy body (thin arrows). Lewy neurites (thick arrows) are also immunopositive. Scale bar, 20 \( \mu \)m. (B) Pigmented nerve cell with two \( \alpha \)-synuclein-positive Lewy bodies. Scale bar, 8 \( \mu \)m. (C) \( \alpha \)-Synuclein-positive, extracellular Lewy body. Scale bar, 4 \( \mu \)m (from [3]).
disease, antibodies directed against the microtubule-binding region of tau protein show a similar behaviour, in that they strongly stain the neurofibrillary lesions, but fail to decorate isolated tau filaments [19].

Future studies with additional α-synuclein antibodies of known epitope will test the validity of the proposed arrangement of α-synuclein in Lewy body filaments. Meanwhile, in conjunction with the discovery of mutations in α-synuclein in some familial cases of Parkinson’s disease [6,44], these findings suggest that the presence of α-synuclein filaments may be the cause of nerve cell death and that idiopathic Parkinson’s disease and dementia with Lewy bodies are α-synucleinopathies.

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**Polyglutamine expansion and Huntington’s disease**

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Huntington’s disease (HD) is an autosomal dominant progressive neurodegenerative disease. Onset is generally in mid life but can range from early childhood to very old age and the duration of the illness is of the order of 15 to 20 years. The symptoms are complex and variable with recognized emotional, motor and cognitive components. The motor disorder can differ markedly between the adult and juvenile forms of the disease. Adult onset HD frequently presents with chorea and dystonia and may progress to an akinetic state. The juvenile patients may never show chorea and are more likely to exhibit a Parkinson-like rigidity, myoclonus, tremor, and suffer from epileptic seizures [1]. HD is classically associated with neuronal cell loss in the caudate nucleus and putamen and in the pyramidal layers of the cortex. The neuropathological changes that underlie HD have been classified into five grades that progress from grade 0, in which there is no gross or microscopic atrophy to the caudate nucleus, to grade 4, in which the most extreme atrophy is observed [2]. Irrespective of grade, a 30% reduction in brain weight has been noted in HD associated with a 20–30% areal reduction across all structures studied [3].

The HD gene spans approximately 180 kb, contains 67 exons and encodes a novel protein of 348 kDa [4,5]. HD is caused by the expansion of a CAG repeat that is located within exon 1 and encodes a polyglutamine (polyQ) tract. In the unaffected population the repeat has a size range of (CAG)0–39 repeat units [6,7], indicating that the normal protein can tolerate a very large variation in the length of the polyQ tract. The expanded size range extends from (CAG)43 to (CAG)130 repeats [8,9]. The vast majority of adult onset HD patients have expansions ranging from (CAG)46 to (CAG)55 repeats. Tracts of (CAG)70 or more invariably cause the juvenile form of the

Abbreviations used: HD, Huntington’s disease; polyQ, polyglutamine; DRPLA, dentatorubral pallidoluysian atrophy; SCA, spinocerebellar ataxia; NI, neuronal intranuclear inclusion; GST, glutathione-S-transferase; LANP, leucine-rich acidic nuclear protein.

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