α-Synuclein Inclusions in Alzheimer and Lewy Body Diseases

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Abstract. α-Synuclein has assumed particular neuropathological interest in the light both of its identification as a non-β-amyloid plaque constituent in Alzheimer disease (AD), and the recent association between dominant inheritance of Parkinson disease (PD) and 2 missense mutations at positions 30 and 53 of the synuclein protein. We report a systematic study of α-synuclein, tau, and ubiquitin immunoreactivity in representative neurodegenerative disorders of late life. The α-synuclein association with Lewy bodies is variable, peripheral, and is not stable with respect to proteases or acid treatment, whereas there is no association with Pick bodies. Stable patterns of immunoreactivity included neurites and a novel inclusion body. Although there is an overlap between the presence of Lewy bodies and stable α-synuclein immunoreactivity, this is seen only in the presence of concomitant neuropathological features of AD. The novel α-synuclein inclusion body identified in pyramidal cells of the medial temporal lobe in particular was found in AD and in the Lewy body variant of AD, and was associated neither with ubiquitin nor tau protein. The inclusion is therefore neither a Lewy body nor a PHF-core body, but may be confused with the Lewy body, particularly in the Lewy body variant of AD. Abnormal processing of α-synuclein leading to its deposition in the form of proteolytically stable deposits is a particular feature of the intermediate stages of AD.

Key Words: Alzheimer disease; Amyloid deposits; α-Synuclein; Cortical Lewy body disease; Neurofibrillary changes.

INTRODUCTION

Alzheimer disease (AD) is characterized by the classical hallmarks, plaques and neurofibrillary tangles, as well as extensive neuronal loss, alterations of the neuronal cytoskeleton, and widespread synaptic loss found in both allocortical and neocortical brain regions. The extent of cortical synaptic loss correlates with the degree of cognitive impairment in AD, and loss of synaptic proteins occurs in both neocortical areas and (para)hippocampal gyrus (1–6). Several presynaptic terminal proteins have been identified within neuritic plaques, including amyloid protein precursor (APP; 7, 8), synaptophysin, synaptobrevin, synapsins and synaptotagmin (for review see 9 and 10). A fragment of another synaptic protein, synuclein, has also been found in amyloid preparations from AD brain tissue and is associated with fibrillary β-amyloid deposits (11).

Synuclein is a 19 kDa protein, located in the perinuclear region and in presynaptic terminals (12) and its expression is developmentally regulated (13, 14). Three synuclein isoforms have been identified to date, with a molecular mass between 16 and 20 kDa. They share 95% homology in the first 110 amino acids from the amino-terminus, which is considered to be crucial for synuclein function (12, 15). A recent study reported sequences for 2 human brain proteins with 140 and 134 amino acid residues (16), of which the longer form (α-synuclein) is identical with the precursor of the non-amyloid component of β-amyloid in AD (11). The 134 amino acid protein (β-synuclein) is the human homologue to bovine phosphonuromyase 14 (17, 18). These 2 synuclein isoforms have similar distribution in both rat and human brain tissue: they are abundant in the neocortical gray matter (16, 18, 19), while in the hippocampal region they are predominantly found in the CA3 layer (16).

Synuclein has assumed particular neuropathological interest in the light both of earlier reports that synuclein may be a non-amyloidogenic precursor component of senile plaques in AD (11, 20–22) and the more recent findings of 2 missense mutations at positions 30 and 53 of the protein (Ala30Pro and Ala53Thr substitutions) that have been associated with autosomal dominant inheritance of Parkinson disease (PD) in 5 unrelated families (23, 24). Neuropathological studies of synuclein to date have been restricted largely to Cortical Lewy Body disease and PD (25–28), as well as multiple system atrophy (29–32), in which various filamentous nerve cell inclusions have been described immunopositive to α-synuclein. However, only a limited number of studies have analyzed AD, and using antibodies raised against epitopes in the conserved N-terminal domain of the molecule (11). From these studies it has been concluded that the region between residues 61–95 of α-synuclein binds to the hydrophobic region of Aβ protein, and that this binding may be a factor in promoting amyloid aggregation (20, 21, 33). The growing evidence of presence of insoluble aggregates of α-synuclein in the Lewy body...
INCLUSIONS IN AD AND LEWY BODY DISEASES

TABLE 1
Age, Sex, Definite Diagnosis, and Neuropathological Characteristics of Analyzed Cases

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age/years</th>
<th>Sex</th>
<th>Definite diagnosis</th>
<th>Braak stage</th>
<th>AP</th>
<th>N</th>
<th>PB</th>
<th>LB</th>
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<td>91</td>
<td>F</td>
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<td>-</td>
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<td>72</td>
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<td>AD</td>
<td>6</td>
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<td>-</td>
</tr>
<tr>
<td>3</td>
<td>82</td>
<td>F</td>
<td>AD</td>
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<tr>
<td>4</td>
<td>74</td>
<td>M</td>
<td>AD+CLBD</td>
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<td>+</td>
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<td>AD+CLBD</td>
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<td>86</td>
<td>M</td>
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<td>PSP</td>
<td>2</td>
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<td>-</td>
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<td>F</td>
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</tr>
<tr>
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<td>2</td>
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</table>

Abbreviations: AD, Alzheimer disease; AD+CLBD, Lewy body variant of Alzheimer disease; CLBD, Cortical Lewy body disease; MID, Multi-infarct dementia; PSP, Progressive supranuclear palsy; AP, amyloid plaques; N, dystrophic neurites; LB, Lewy bodies; PB, Pick’s bodies; M, male; F, female.

Note: Presence (+) or absence (−) of distinct neuropathological features is included in the table. Distribution of neurofibrillary tangles is indicated via the Braak stage.

MATERIALS AND METHODS

Brain Tissue
The study was performed on human brain tissue obtained from the collection of the Cambridge Brain Bank. Tissue from a total number of 23 subjects was included in the analyses: 7 met the criteria for definite diagnosis of AD (35), 3 had Pick’s disease, 6 had cortical Lewy body dementia (CLBD; 4 of these with coexistent Alzheimer-type pathology and 2 without), 3 were diagnosed as progressive supranuclear palsy (PSP), 1 individual had Down syndrome and AD-type pathological hallmarks, 2 cases had PD (combined with AD in 1 case), 2 cases had multi-infarct dementia (MID), and 3 cases with no cognitive impairment served as the control group (Table 1). The neuropathological diagnosis and the neuroanatomical distribution of neurofibrillary pathology according to Braak and Braak (36) was established by one of us (JX).

Light Microscopy
For light microscopy, immunohistochemistry was performed in 23 cases on sections of medial temporal lobe, containing dentate gyrus, Cornu Ammonis, subicular complex, and entorhinal cortex (Table 1). In addition, sections from nucleus basalis of Meynert from 3 additional AD cases (age at death: 2 cases 70 years and one 76 years) and 1 control individual (age at death 88 years), as well as the olfactory bulb of 1 AD individual.
The visualizations of these conditions were found to be optimal for vi-

Pronase staining, serial sections were incubated before the blocking step

were preincubated with 20
cbind was blocked with 2% fat-free

microliters: a green excitation

The density of neurofibrillary pathology (intra- and extra-
cellular tangles, dystrophic neurites, neuritic plaques) and am-
yloid deposits was determined as described previously (37). The
total count of neurofibrillary tangles represents the sum of the
density of intracellular tangles (immunostained with mAb 7.51) and the density of extracellular tangles (visualized with mAb 423).
The number of diffuse plaques was obtained after deducting the number of neuritic plaques (immunolabelled with mAb 7.51) from the total number of plaques (immunodetected with mAb 9.38).

**Statistical Analysis**

Differences between mean values of different neuropathological features were compared by 2-tailed Student t-test. The non-
parametric Spearman correlation coefficients were calculated when comparing neuropathological lesions with each other. The statistical significance of frequency associations was determined by the Fisher exact test.

**RESULTS**

**Neuropathological Characterization of Cases in the Present Study**

The cases selected for this study spanned a representative range of the neurodegenerative disorders seen in late life according to conventional neuropathological diagnoses, age, and sex (Table 1). One third of the cases examined met either McKhann et al (1984) (35) criteria for a diagnosis of AD or had Lewy bodies. All cases were also staged according to the criteria of Braak and Braak (36). Presence or absence of a range of other characteristic neuropathological lesions, including β-amyloid plaques, dystrophic neurites, and Pick’s bodies have also been estimated quantitatively. Except for cases with Pick bodies, who were younger at death than those without (mean ages 67.5 ± 0.5 and 74.2 ± 2.4 years, respectively, p = 0.012), there were no other significant age differences with respect to presence of any of the neuropathological lesions examined.

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**Table 2**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dilution</th>
<th>Treatment Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAb 423 (anti-tau)</td>
<td>1:5</td>
<td>— ECT</td>
</tr>
<tr>
<td>mAb 7.51 (anti-tau)</td>
<td>1:500</td>
<td>— ICT, ECT, NP, N</td>
</tr>
<tr>
<td>mAb 11.57 (anti-tau)</td>
<td>1:5</td>
<td>— ICT, ECT, NP, N</td>
</tr>
<tr>
<td>mAb EP10 (anti-synaptophysin)</td>
<td>1:10</td>
<td>— DP, NP, burnt-out plaques</td>
</tr>
<tr>
<td>pAb BR251 (anti-ubiquitin)</td>
<td>1:50</td>
<td>— ICT, NP, N</td>
</tr>
<tr>
<td>pAb PER2 (anti-α synuclein)</td>
<td>1:50</td>
<td>Formic acid, Pronase NP, N, diffuse neuronal staining, neuronal inclusions</td>
</tr>
</tbody>
</table>

Abbreviations: ECT, extracellular neurofibrillary tangles; ICT, intracellular neurofibrillary tangles; NP, neuritic plaques; DP, diffuse plaques; N, dystrophic neurites.

(although at death 74 years), not listed in the table, were also immunostained for synuclein protein. Serial sections of 10 μm

thickness were cut from paraffin embedded blocks, fixed in methanol acetic acid (95/5 v/w). The sections were deparaffin-
ized, and nonspecific binding was blocked with 2% fat-free

milk/PBS and immunostained as described previously (37) with antibodies as stated in Table 2. For the α-synuclein immuno-

staining, serial sections were incubated before the blocking step with: (i) 100% formic acid for 5 min; or (ii) 1 mg/ml Pronase

for 2 min. These conditions were found to be optimal for vi-

ralization of α-synuclein immunoreactivity, and there were no

quantitative differences in the number of visualized immuno-

reactive features between these 2 methods. The quantitative
data reported in the present study were obtained on sections

immunostained following Pronase treatment. The formic acid

pretreated sections were used for double labelling, since the

epitopes of some of the antibodies used in the present study (such as BR251 and 11.57) are abolished after protease diges-

tion. For α-synuclein immunohistochemistry, sections were also

incubated without the primary antibody. When PER2 antisemur was preincubated with 20 μg of the synthetic peptide that the

serum was raised against, the

antibodies (all used at full strength) for 30 min at room tempera-
ture, followed by 30 min incubation with a mixture of fluoro-

chrome-labelled secondary antibodies. The mAbs 423, 7.51, 11.57, 9.38 and pAb BR251 were labelled with FITC-conju-
gated goat immunoglobulins to mouse globulins (1:40; Sigma

Immunoclochemichal Co., St Louis, MO). For PER2 serum, bio-
tinylated anti-rabbit immunoglobulin was used, followed by TexasRed avidin (used in 1:40; Vector Laboratories, Peterbor-
ough, UK). Sections were washed in PBS and mounted on glass

slides in Vectashield mounting medium (Vector Laboratories, Inc., Burlington, CA). Selected microscopic fields were

scanned with an MRC-600 confocal microscope imaging sys-
tem (Bio-Rad, Cambridge, MA). The system was equipped with

an argon laser and high sensitivity filters: a green excitation

filter set containing 568 nm exciter filter, and a blue excitation filter set containing 488 nm. The emission spectra from the

excited fluorophores were passed to photomultipliers, viewed on a monitor and photographed.
As expected, cases meeting the McKhann criteria for a neuropathological diagnosis of AD were at significantly more advanced Braak stages than those that did not meet the criteria (4.2 ± 0.5 vs 1.4 ± 0.4, p = 0.0001). Although agreement with McKhann criteria was maximized at the cut-off of Braak stage 4 or greater (87% agreement, p = 0.001), a third of the cases meeting the McKhann criteria were at Braak stages 3 or less. As expected, there were significant associations between a neuropathological diagnosis of AD by either criteria (McKhann or Braak stage) and presence of amyloid plaques and dystrophic neurites, and cases with these lesions were at significantly more advanced Braak stages than those without. In contrast, there were no significant associations between presence of Lewy bodies in cortex and/or brainstem and neuropathological diagnosis of AD by either criteria and in this series cases did not differ in mean Braak stage according to presence or absence of Lewy bodies (2.6 ± 0.6 vs 2.5 ± 0.6 respectively, p = 0.919).

**Immunohistochemical Features Detected with Anti-α-synuclein in Hippocampus and Entorhinal Cortex**

Without pretreatment of sections with either Pronase (1 mg/ml, 2 min) or formic acid (100%, 5 min), no consistent pattern of immunolabelling could be detected in medial temporal lobe structures with the C-terminal α-synuclein antiserum in any of the diagnostic categories examined, including PD and the Lewy body variant of AD. However, following either of these treatments, 4 consistent patterns of immunolabelling could be identified: (i) diffuse intraneuronal labelling (Fig. 1a–c), (ii) neuritic plaques (Fig. 1d, f), (iii) neuritic processes (Fig. 1e, g), and (iv) dense inclusion-like structures within pyramidal cells (Fig. 1h–k).

**Diffuse Labelling:** All but 3 cases (87%) showed evidence of diffuse intraneuronal labelling of either hippocampus (CA2/3 region) or entorhinal cortex (Fig. 1a–c). There was no significant association between presence of this feature and any neuropathological diagnosis or with any other of the neuropathological features examined. Labelling was seen in entorhinal cortex in fewer cases than in hippocampus, and the mean density of cells labelled was also significantly less than in hippocampus (t = 2.36, p = 0.023).

**Neuritic Plaques:** Neuritic plaques containing sparse α-synuclein immunoreactivity were found in 5 cases, 4 of them meeting McKhann criteria for a neuropathological diagnosis of AD (Fig. 1d, f). Synuclein-immunoreactive plaques were found in CA3 region of hippocampus and the outer layers of the entorhinal cortex. There were no clear differences between entorhinal cortex and hippocampus in terms of either frequency of cases with labelled plaques or counts of labelled plaques (t = 0.66, p = 0.527). Labelled neurites were both sparser and more peripheral than the tau-immunoreactive neurites characteristically seen in plaques. There was no evidence of colocalization of α-synuclein and tau-immunoreactivity in neuritic plaques, and plaque cores were unlabelled.

**Neurites:** Neurites immunoreactive for α-synuclein were detected in the neuropil of 6 cases; all of them meeting McKhann criteria for a diagnosis of AD (Fig. 1e, g). Whereas the association between presence of α-synuclein positive neurites and neuropathological diagnosis of AD was significant (87% agreement, p < 0.0001), this was not the case when Braak staging criteria were implemented: cases with neuritic labelling in the neuropil were not at more advanced Braak stages. There was, however, a significant association between neuritic α-synuclein immunoreactivity and presence of Lewy bodies (83% agreement, p = 0.009).

More neuritic labelling was found in hippocampus than in entorhinal cortex (t = 3.40, p = 0.007) and the labelling of neurites was widespread in 2 cases with pure AD. Although tau- and ubiquitin-immunoreactive dystrophic neurites, containing ubiquitin and tau protein could also be detected in both of these cases, their morphology and topographical localization differed. Thus, while tau- and ubiquitin-reactive neurites were found predominantly in the stratum pyramidale of hippocampus, the α-synuclein-reactive neurites were found predominantly within the stratum oriens of the CA3 and CA2 region of hippocampus, but rarely in stratum lucidum or the outer layers of CA3 stratum pyramidale. Likewise, subiculum and CA4 region contained few α-synuclein-positive neurites. In entorhinal cortex, α-synuclein-reactive neurites were largely restricted to layer II.

**Dense Inclusions:** Densely labelled spherical inclusions within pyramidal cells were found in 7 cases (Fig. 1h–k), all of them meeting the McKhann criteria for diagnosis of AD. This association was significant (91% agreement, p < 0.001). There was a lower, but nevertheless significant, level of agreement with a diagnosis of AD by Braak staging criteria (78% agreement, p = 0.045), and cases with dense inclusions were at significantly more advanced Braak stages than those without (BST 3.9 ± 0.6 vs 1.7 ± 0.5, p = 0.014). The association with presence of Lewy bodies was of a similar order and likewise significant (78% agreement, p = 0.026).

In medial temporal lobe structures, pyramidal cells containing dense inclusions were located in CA3 and CA2 of hippocampus, and in layers II and IV of entorhinal cortex. The density of these inclusions was somewhat higher in the entorhinal cortex in comparison with density detected in the hippocampal gyrus (t = 1.82, p = 0.094). Abundant inclusions and widespread neuritic labelling were also found in nucleus basalis of Meynert (3 AD and 1 elderly control cases; Fig. 1e) and in olfactory bulb (1 AD case).
Confocal Immunohistochemistry of Dense α-synuclein Inclusions

We used double-labelling confocal microscopy to determine the immunohistochemical relationship between α-synuclein immunoreactive inclusions and other neuropathological inclusions with which they might be confused. Despite the strong statistical associations with a pathological inclusions with which they might be confused, α-synuclein inclusions were typically found in pyramidal cells of normal histological appearance, and were never detected in tangle-bearing neurons (Fig. 1h–k). We found no evidence of α-synuclein labelling of Pick bodies, which were nevertheless labelled both by anti-tau and anti-ubiquitin antibodies (data not shown). As expected, cortical Lewy bodies showed dense and uniform anti-ubiquitin labelling (Fig. 1l). In contrast, labelling by anti-α-synuclein was restricted to variable aggregates located at the periphery of occasional Lewy bodies, with little evidence of overlap with ubiquitin immunoreactivity (Fig. 1l). Conversely, the dense α-synuclein inclusions were unlabelled by anti-ubiquitin or anti-tau antibodies (Fig. 1m). Furthermore, the size and texture of labelling of Lewy bodies and the dense α-synuclein inclusions differed. Whereas Lewy bodies were typically more than 50 μm in diameter and with relatively uniform texture, α-synuclein inclusions were less than 30 μm in diameter and were more coarsely granular and with a more irregular outline (Fig. 1l, m).

Relationship between α-synuclein Pathology and Lewy Body Disease

The 4 cases with presence of Lewy bodies in either neocortex or brainstem that had both α-synuclein inclusions and immunoreactive neurites in the neuprol met the neuropathological diagnosis of AD by McKhann criteria. The cases with cortical Lewy Body disease that were devoid of α-synuclein pathology (inclusions and neurites) had fewer intracellular tangles, no extracellular neurites, no diffuse β-amyloid or tau-reactive neuritic plaques, and widespread accumulation of diffuse α-synuclein staining within the pyramidal neurons, confined to the hippocampal region only.

As the α-synuclein inclusions occurred in individuals meeting the McKhann’s criteria for AD, we attempted to characterize the relationship with neuropathological features of the disease further. Of the cases meeting the McKhann criteria for a diagnosis of AD, those with α-synuclein inclusions had significantly fewer tangles (t = 3.08, p = 0.009) and significantly shorter duration of disease (t = 3.07, p = 0.018) than those without (Fig. 2). In contrast, the density of β-amyloid plaques did not differ between the 2 groups. AD cases with α-synuclein inclusions also had fewer ubiquitin-reactive plaques (t = 3.33, p = 0.005) and fewer pyramidal cells with diffuse synuclein immunoreactivity (t = 2.36, p = 0.035). Although the tendency for AD cases with α-synuclein inclusions to be at relatively earlier Braak stages did not achieve statistical significance (3.91 ± 1.2 vs 5.5 ± 0.5, p = 0.100), counts of pyramidal cells containing α-synuclein inclusions showed a strong association with intermediate stages of pathology (Fig. 3), with progressive disappearance of the inclusions in medial temporal lobe structures at more advanced neuropathological stages.

This inverse relationship between the severity of AD pathology and α-synuclein immunoreactivity could also be seen from correlation analysis (Table 3). There were significant inverse relationships between β-amyloid plaques and α-synuclein immunoreactivity detected both in plaques (r = −0.53, p = 0.044) and in the neuropil (r = −0.78, p = 0.001). There was also an inverse relationship between α-synuclein immunoreactivity and synaptophysin immunoreactivity in plaques (r = −0.49, p = 0.049). Although all the relationships between tau- and α-synuclein pathology were inverse, only that between counts of cells with α-synuclein inclusions and tau-reactive neuritic plaques achieved statistical significance (r = −0.59, p = 0.045).

DISCUSSION

Human α-synuclein is a highly conserved 140 amino-acid protein of unknown function that is expressed abundantly in nervous system, and is particularly concentrated...
Fig. 2. Density of neurofibrillary changes and amyloid deposits in cases with definite diagnosis of AD with (AD+; n = 7) and without (AD−; n = 2) intraneuronal synuclein containing inclusions. The figures in each AD group derive from data points from entorhinal cortex and hippocampus. The total density of neurofibrillary tangles (NFT) refers to the sum of density of intracellular (ICT, visualized with mAb 7.51) and extracellular (ECT, immunostained with mAb 423) tangles. The density obtained after deducting the neuritic plaque counts (NP, immunolabelled with mAb 7.51) from the density of the total amyloid plaques (AP; immunostained with mAb 9.38) was used as a measure of the density of diffuse plaques (DP) (**, p < 0.01).

in presynaptic nerve terminals (16). Two distinct lines of neuropathological investigation have converged on the potential role of α-synuclein in neurodegenerative disease. Synuclein was found to be a non-APP-derived precursor or constituent of β-amyloid plaques in AD (11, 20–22) and appears therefore to represent one of what may become a growing list of non-APP precursors or associated proteins found within the senile plaques in AD. The more recent discovery that a mutation localized to the α-synuclein gene segregates with certain familial forms of PD (23, 24), and evidence for α-synuclein immunoreactivity in Lewy bodies (25–28), has focused attention on a significant involvement of this protein in the molecular neuropathology of PD and related neurodegenerative disorders. The potential overlap between AD and Lewy body disease had already become the object of neuropathological enquires since the discovery that anti-ubiquitin and anti-APP antibodies could be used to detect cortical Lewy bodies (8, 38) in a far higher proportion of cases coming to postmortem with clinical and neuropathological features of AD than had been suspected previously, and the evidence that genetic mutations in the amyloid precursor protein could be expressed phenotypically either in terms of a characteristic AD-type or cortical Lewy body-type neuropathological picture (39–42). These lines of evidence therefore bring together α-synuclein, tau protein, β-amyloid protein, and ubiquitin in a range of neurodegenerative disorders whose diagnostic boundaries remain for the present indistinct.

In light of these uncertainties, we have undertaken a quantitative immunohistochemical study of α-synuclein neuropathology in a representative sample of the commoner forms of neurodegenerative disorder of late life, with particular reference to the overlap between AD and diseases characterized by the presence of Lewy bodies in cortex or brainstem. We report that it is possible to identify 4 distinct patterns of α-synuclein immunoreactivity consistently in histological sections pretreated with either formic acid or Pronase. These patterns include diffuse cytoplasmic labelling, labelling of neurites within plaques or diffusely scattered in the neuropil, and labelling of dense synuclein-immunoreactive inclusions within pyramidal cells found in medial temporal lobe structures. Of these, only α-synuclein-immunoreactive neurites and dense inclusions showed any consistent association with either conventional or operational neuropathological diagnosis. Both were associated in a statistically significant manner with AD and diseases characterized by the presence of Lewy bodies in either neocortex or brainstem. These results therefore confirm that there is a statistically robust association between a restricted subset of recognized neuropathological diagnosis and some as yet unknown pathological processing of the C-terminal segment of the α-synuclein molecule, which is expressed in terms of 2 characteristic immunohistochemical profiles.

When the cases with Lewy body pathology were examined further, there was a significant association between the neuritic and dense inclusion α-synuclein immunoreactivity and presence of Lewy bodies. However, the Lewy body dementia group appeared to be somewhat heterogeneous, with 2 of 6 individuals examined having only diffuse intracellular α-synuclein staining in the medial temporal lobe. These cases had less neurofibrillary...
pathology and were devoid of amyloid extracellular deposits. These findings raise a possibility that the differences in the extent of α-synuclein pathology within the Lewy body dementia spectrum may be explained by co-existent AD. In the light of the small sample analyzed in the current study, these findings will need to be explored further and confirmed in a larger sample size.

We were also unable to confirm any consistent labeling of Lewy bodies by the same C-terminal α-synuclein antiserum as that previously reported to label these structures (25, 26). Although there was an occasional association between Lewy bodies immunolabelled with anti-ubiquitin and intracellular inclusions immunoreactive with the α-synuclein antiserum, this was variable and peripheral to the Lewy body itself. Therefore, despite the genetic data linking a mutation in the α-synuclein molecule into the Lewy body, it appears not to the form either of integral incorporation of the α-synuclein molecule into the Lewy body. A most recent study also failed to detect α-synuclein labeling of Lewy bodies in an individual without overt signs of dementia or parkinsonism (43).

On the other hand, we report the existence of a novel pathological inclusion characterized by dense α-synuclein immunoreactivity that may perhaps be confused with the Lewy body. The 2 are, however, immunochemically and morphologically distinct. Thus, whereas the Lewy body is characterized by strong ubiquitin immunoreactivity, the α-synuclein inclusion is not. Furthermore, the Lewy body is larger and more homogeneous in texture than the α-synuclein inclusion we have described. The α-synuclein inclusion is also distinct from the 2 other pathological inclusions, which can be found within pyramidal cells in AD, namely the granulo-vacuolar degeneration complex (44) and the PHF-core body (45), that have distinct morphology (GVD) and location (PHF-core body). Furthermore, these structures are tau-immunoreactive whereas the α-synuclein inclusion is not. On the other hand, the strong statistical association between the α-synuclein inclusion and an independent pathological feature taking the form of α-synuclein-immunoreactive neurites suggests that both are characteristic markers of an as yet unidentified molecular abnormality in the processing of at least the C-terminal segment of the α-synuclein molecule.

Although the features of α-synuclein pathology that we have described are characterizedly associated with a neuropathological diagnosis of AD and Lewy body dementia with coexistent Alzheimer-type pathology, there does not appear to be any simple or direct link with abnormal processing of either the β-amyloid precursor protein or tau protein. Thus, there is an inverse relationship between β-amyloid plaques and α-synuclein-immunoreactive neurites found either in plaques or in the neuropil. Likewise, the α-synuclein inclusions are inversely related to the quantity of tau-immunoreactive

### TABLE 3

<table>
<thead>
<tr>
<th>Synuclein staining/</th>
<th>Neurites</th>
<th>Plaques</th>
<th>Cells</th>
<th>Inclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICT (mAb 7.51)</td>
<td>0.230</td>
<td>0.06</td>
<td>0.49</td>
<td>−0.27</td>
</tr>
<tr>
<td></td>
<td>(0.420)</td>
<td>(0.839)</td>
<td>(0.073)</td>
<td>(0.349)</td>
</tr>
<tr>
<td>ECT (mAb 423)</td>
<td>−0.22</td>
<td>0.22</td>
<td>0.01</td>
<td>−0.13</td>
</tr>
<tr>
<td></td>
<td>(0.406)</td>
<td>(0.395)</td>
<td>(0.982)</td>
<td>(0.631)</td>
</tr>
<tr>
<td>Total tangle counts</td>
<td>0.30</td>
<td>0.20</td>
<td>0.44</td>
<td>−0.22</td>
</tr>
<tr>
<td></td>
<td>(0.301)</td>
<td>(0.495)</td>
<td>(0.120)</td>
<td>(0.448)</td>
</tr>
<tr>
<td>Total plaque counts</td>
<td>−0.78</td>
<td>−0.53</td>
<td>−0.14</td>
<td>−0.28</td>
</tr>
<tr>
<td>(mAb 9.38)</td>
<td>(0.001)</td>
<td>(0.044)</td>
<td>(0.614)</td>
<td>(0.312)</td>
</tr>
<tr>
<td>Diffuse plaques</td>
<td>−0.79</td>
<td>−0.26</td>
<td>−0.12</td>
<td>−0.07</td>
</tr>
<tr>
<td></td>
<td>(0.001)</td>
<td>(0.396)</td>
<td>(0.698)</td>
<td>(0.815)</td>
</tr>
<tr>
<td>Neuritic plaques</td>
<td>0.21</td>
<td>−0.10</td>
<td>0.30</td>
<td>−0.49</td>
</tr>
<tr>
<td>(mAb 7.51)</td>
<td>(0.465)</td>
<td>(0.726)</td>
<td>(0.303)</td>
<td>(0.077)</td>
</tr>
<tr>
<td>Synaptophysin plaque</td>
<td>−0.04</td>
<td>−0.49</td>
<td>0.001</td>
<td>−0.17</td>
</tr>
<tr>
<td>(mAb EP10)</td>
<td>(0.880)</td>
<td>(0.049)</td>
<td>(0.992)</td>
<td>(0.523)</td>
</tr>
<tr>
<td>Dystrophic neurites</td>
<td>−0.08</td>
<td>0.11</td>
<td>0.23</td>
<td>−0.37</td>
</tr>
<tr>
<td>(mAb 11.57)</td>
<td>(0.758)</td>
<td>(0.675)</td>
<td>(0.383)</td>
<td>(0.149)</td>
</tr>
</tbody>
</table>

**Note:** The analysis refers to the entorhinal cortex and hippocampus from 9 cases with definite diagnosis of AD. The antibodies that have been used for immunolabelling distinct neuropathological structures are shown in brackets. The number of diffuse plaques was obtained after deducting the number of neuritic plaques (immunolabelled with mAb 7.51) from the total number of plaques (immunodetected with mAb 9.38). The total tangle counts is the sum of density of intracellular and extracellular neurites (immunolabelled with mAb 7.51). The total plaque counts is the sum of density of intracellular and extracellular neurofibrillary tangles detected with mAb 7.51 and mAb 423, respectively. The numbers in the table refer to the coefficient of correlation, whereas the figures within the brackets below denote the corresponding p value.

**Abbreviations:** ICT, intracellular neurofibrillary tangles; ECT, extracellular neurofibrillary tangles.

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neuritic plaques. We found no evidence of colocalization of α-synuclein and tau immunoreactivity either in terms of preponderant neuroanatomical localization within the medial temporal lobe structures or within individual dystrophic neurites. Whereas the characteristic tau- and β-amyloid-immunoreactive pathological features increase with disease progression, the α-synuclein pathology appears to be a feature of the middle stages of AD as defined by Braak and Braak (36).

The accentuation of α-synuclein pathology occurs in the limbic stages of tangle propagation, and in another independent study we also found increased levels of soluble α-synuclein, measured biochemically, at the same disease stage in the human neocortex (EBM-L, unpublished data). Similarly, Iwai et al (46) described slightly increased levels of immunoreactivity for synuclein in frontal lobe at early AD. It is of interest to note that the increased expression of α-synuclein in the medial temporal lobe coincides neuroanatomically with the transient dendritic sprouting confined to this region in middle stages of AD (47). At present, one can only speculate about the possible induction mechanism underlying the increased α-synuclein expression in the limbic stages of AD. Numerous trophic factors have been identified which mediate neuronal sprouting responses to neuronal injury and partial de-afferentation (48, 49) and many of them are found within the corona of neuritic plaques prior to the appearance of phosphorylated tau protein (50). Further research on a larger number of well-documented individuals covering the full neuroanatomical and clinical spectrum of AD will be required to determine the clinical and biological relevance of this finding.

In summary, we report the existence of a novel neuropathological inclusion found within pyramidal cells of medial temporal lobe structures at intermediate stages of AD. This inclusion is characterized by dense immunoreactivity associated with the C-terminal segment of the α-synuclein molecule, which is closely associated with the appearance of α-synuclein-immunoreactive neurites in the neuropil. Although there appears to be some overlap with the presence of Lewy bodies both diagnostically and at the histological level, we were unable to confirm the integral incorporation of the C-terminal tail of α-synuclein within the Lewy body itself. The α-synuclein pathology in the form of neuritic plaques, neurites, and dense inclusions, appears in the presence of concomitant AD pathology. Therefore, the molecular pathophysiology of abnormal processing of α-synuclein that may be a consequence of the mutations that have recently been linked genetically with familial PD does not appear to be via the straightforward incorporation of this molecule into the structures that are pathognomonic of the disease. Rather, we are left with accounting for the involvement of a range of different molecules in partially overlapping patterns of neurodegenerative disease, which as yet defy any attempt at unified theoretical explanation at the molecular level.

ACKNOWLEDGMENTS

We are indebted to Angela O’Sullivan for liaison with the carers of the deceased and Richard Hills for technical support. This study was supported by an MRC grant, UK and Leopold Muller Estate. WGH was supported by MRC Canada. EBM-L is a Research Fellow of Hughes Hall, University of Cambridge.

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Received August 11, 1999
Revision received January 26, 2000
Accepted February 9, 2000