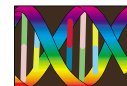
ORIGINAL CONTRIBUTION

α-Synuclein in Familial Alzheimer Disease



Epitope Mapping Parallels Dementia With Lewy Bodies and Parkinson Disease

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Background: α-Synuclein is a major component of Lewy bodies (LBs) in Parkinson disease and dementia with LBs and of glial cytoplasmic inclusions in multiple system atrophy. However, epitope mapping for α-synuclein is distinctive in different neurodegenerative diseases. The reasons for this are poorly understood but may reflect fundamental differences in disease mechanisms.

Objective: To investigate the α-synuclein epitope mapping properties of LBs in familial Alzheimer disease.

Design and Setting: We compared LBs in familial Alzheimer disease with those in synucleinopathies by probing 6 brains of persons with familial Alzheimer disease using a panel of antibodies to epitopes spanning the α-synuclein protein. Results were compared with data from brains of persons with Parkinson disease, dementia with LBs, and multiple system atrophy.

Results: The brains of persons with familial Alzheimer disease showed consistent staining of LBs with all antibodies, similar to Parkinson disease and dementia with LBs but different from α-synuclein aggregates that occurred in multiple system atrophy.

Conclusions: These data suggest that the epitope profiles of α-synuclein in LBs are similar, regardless of whether the biological trigger is related to synuclein or a different genetic pathway. These findings support the hypothesis that the mechanism of α-synuclein aggregation is the same within cell types but distinctive between cell types.

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Although α-synuclein aggregates are traditionally associated with synucleinopathies such as Parkinson disease (PD) and dementia with Lewy bodies (DLB), they also occur in Alzheimer disease (AD) and Down syndrome.[1-3] In particular, most symptomatic patients with mutations of the presenilin (*PS*) or amyloid precursor protein (*APP*) genes have Lewy bodies (LBs) in the amygdala and adjacent entorhinal cortex.[2] This suggests that the genetic mechanism that leads to β-amyloid plaque and tau-rich neurofibrillary tangle formation in AD with *PS* and *APP* mutations also predisposes individuals to LBs formed by α-synuclein filaments.

Questions remain regarding the exact mechanism of α-synuclein aggregation in neurodegenerative diseases. In PD and DLB, full-length α-synuclein is present in filamentous LBs, and they are strongly immunoreactive for antibodies that recognize epitopes along the entire protein.[4] In multiple system atrophy (MSA), a condition in which filamentous α-synuclein aggregates form glial cytoplasmic inclusions (GCIs), α-synuclein epitope mapping is not uniform.[4] Therefore, structural or conformational differences in aggregated α-synuclein exist in the filamentous α-synuclein lesions of different diseases. This suggests that mechanisms leading to α-synuclein aggregation in LBs vs GCIs are not identical.

Most epitope-mapping studies of α-synuclein have focused on synucleinopathies. It is unknown whether LB formation in AD occurs through a distinct mechanism or whether all LBs result from identical responses to different triggers. Since analysis of the underlying aggregated protein has the potential to yield clues regarding the mechanism of inclusion formation, the current study evaluates LB formation in early-onset familial AD (FAD), in which disease etiology is not primarily related to an abnormality of the α-synuclein gene.

RESULTS

Using the same LB509 Mab as in prior studies,[2-4] we confirmed that there is robust staining of LBs and Lewy neurites in sections of amygdala and the adjacent entorhinal cortex in FAD. Using the other

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MATERIALS AND METHODS

TISSUE SAMPLES

We examined affected regions from 6 patients with FAD who were known to have LBs, including 5 patients with *PS-1* mutations and 1 patient with an *APP* mutation (**Table**).[2] All had LBs in the amygdala, and 4 (patients 1 and 4-6) also had LBs in the entorhinal cortex, cingulate gyrus, frontal cortex, nucleus basalis of Meynert, substantia nigra, and locus coeruleus. Although disease duration was variable, all patients were in nursing homes, requiring assistance with all activities of daily living at the time of death. Tissue samples for the current analysis were derived from the amygdala and adjacent entorhinal cortex in all cases, and also included the other involved brain regions in patient 5. These data were compared with those acquired using the same method from brains of persons with PD, DLB, and MSA.[4]

IMMUNOHISTOCHEMICAL ANALYSIS AND ANTI–α-SYNUCLEIN ANTIBODIES

Tissue blocks were formalin fixed, paraffin embedded, and serially cut at 6 µm. Tissues were then stained with a panel of monoclonal antibodies (Mabs) (SNL-4, SYN204, LB509, 211, 202) (**Figure 1**), described previously,[4] that recognize identified epitopes spanning the α-synuclein protein. Formic acid pretreatment optimized staining,[4] and the topographically distinct α-synuclein epitopes were detected using avidin-biotin complex kits (Vector Laboratories, Burlingame, Calif) and 3,3′-diaminobenzidine.[4] Positive control specimens consisted of LB-rich brain sections from the amygdala of a patient with DLB. Consecutive sections stained with the supernatant from unfused SP2/0-Ag14 mouse myeloma cells in place of primary antibodies were used as negative controls. A previously described[2] semiquantitative scale was used to document the degree of LB staining in the amygdala, where 0 indicates no LBs; 1, 1 to 5 LBs; 2, 6 to 20 LBs; and 3, more than 20 LBs. Since LBs were numerous in several of these cases, we added an additional category (grade 4) where there were more than 20 LBs per microscopic field (magnified ×20).

Clinical Features and Epitope Mapping in the Amygdala of Patients With Familial Alzheimer Disease*

Patient No./Sex/Age at Death, y	Site of Mutation	Disease Duration, y	Lewy Body Grade, Monoclonal Antibody				
			SNL-4	SYN204	LB509	211	202
1/F/54	APP 717	5	4	4	4	4	4
2/F/60	PS1 C410Y	15	4	4	4	4	4
3/F/66	PS1 A246E	23	4	4	4	4	4
4/F/61	PS1 M146L	8	4	4	4	4	4
5/M/51	PS1 M146L	6	4	4	4	4	4
6/M/61	PS1 L286V	8	4	4	4	4	4

*All patients were white. Mean age at death was 58.8 years; mean duration of disease, 10.8 years. Grading system for Lewy bodies is described in the "Immunohistochemical Analysis and Anti–α-Synuclein Antibodies" subsection of the "Materials and Methods" section. APP indicates amyloid precursor protein; PS1, presenilin 1; C410Y, cysteine-to-tyrosine substitution at nucleotide 410; A246E, alanine–to–glutamic acid substitution at nucleotide 246; M146L, methionine-to-leucine substitution at nucleotide 146; and L286V, leucine-to-valine substitution at nucleotide 286.

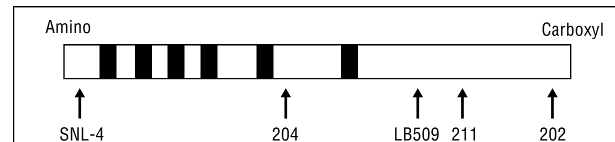

Figure 1. A diagram of the α-synuclein protein indicating the approximate sites where the α-synuclein antibodies are directed (arrows). Monoclonal antibody SNL-4 targets α-synuclein amino acids 2 through 12; SYN204, α-synuclein amino acids 87 through 110 (hydrophobic region); LB509, α-synuclein amino acids 115 through 122; 211, α-synuclein amino acids 121 through 125; and 202, α-synuclein amino acids 130 through 140.

Mabs that recognize epitopes spanning the protein (**Figure 2**A-E), we observed a similar pattern of LB staining in FAD. In particular, the SNL-4 Mab stained equivalent numbers of LBs as LB509 and the other Mabs, although the intensity of staining was slightly less with SNL-4 than with the other Mabs. The staining pattern was indistinguishable between our patients with DLB (Figure 2F-J) and FAD, except that the LBs in patients with FAD (Figure 2A-E) more often had a sharply irregular, eccentric shape due to the co-occurrence of neurofibrillary tangles in some neurons with LBs. Identical, uniform epitope staining was seen in LBs in other cortical regions. Nigral LBs had a staining pattern similar to those of the substantia nigra in PD.[4] However, the α-synuclein epitope profile of LBs in FAD differed from that of GCIs in MSA, in which a reduced number of cells were immunoreactive for the SNL-4 Mab.[4] We noted no differential patterns of immunoreactivity when comparing FAD with *PS* and *APP* mutations, or FAD with different *PS* mutations, and LB staining was graded 4 with all antibodies for all of our patients with FAD.

COMMENT

We extended previous data on LB formation from filamentous α-synuclein aggregates in FAD by demonstrating that LBs in FAD contain the full-length α-synuclein protein. We showed that the α-synuclein epitope profile was indistinguishable between the LBs in FAD and the LBs in DLB and PD, but that the profile differed from that seen in the GCIs in MSA. Moreover, we found no differential staining pattern for α-synuclein in LBs between different mutations in FAD.

Abnormalities of aggregated α-synuclein are implicated in the pathogenesis of PD, DLB, and MSA. The current data are interesting because they reinforce the notion that the morphologic features of the α-synuclein filaments that form in neurons differ from those of α-synuclein filamentous aggregates in glia. Duda et al[4] determined that the C-terminal epitopes are strongly immunoreactive for α-synuclein in GCIs, whereas N-terminal epitopes show less consistent immunoreactivity. In comparison, LBs in PD and DLB are uniformly immunoreactive for all α-synuclein antibodies. The rea-

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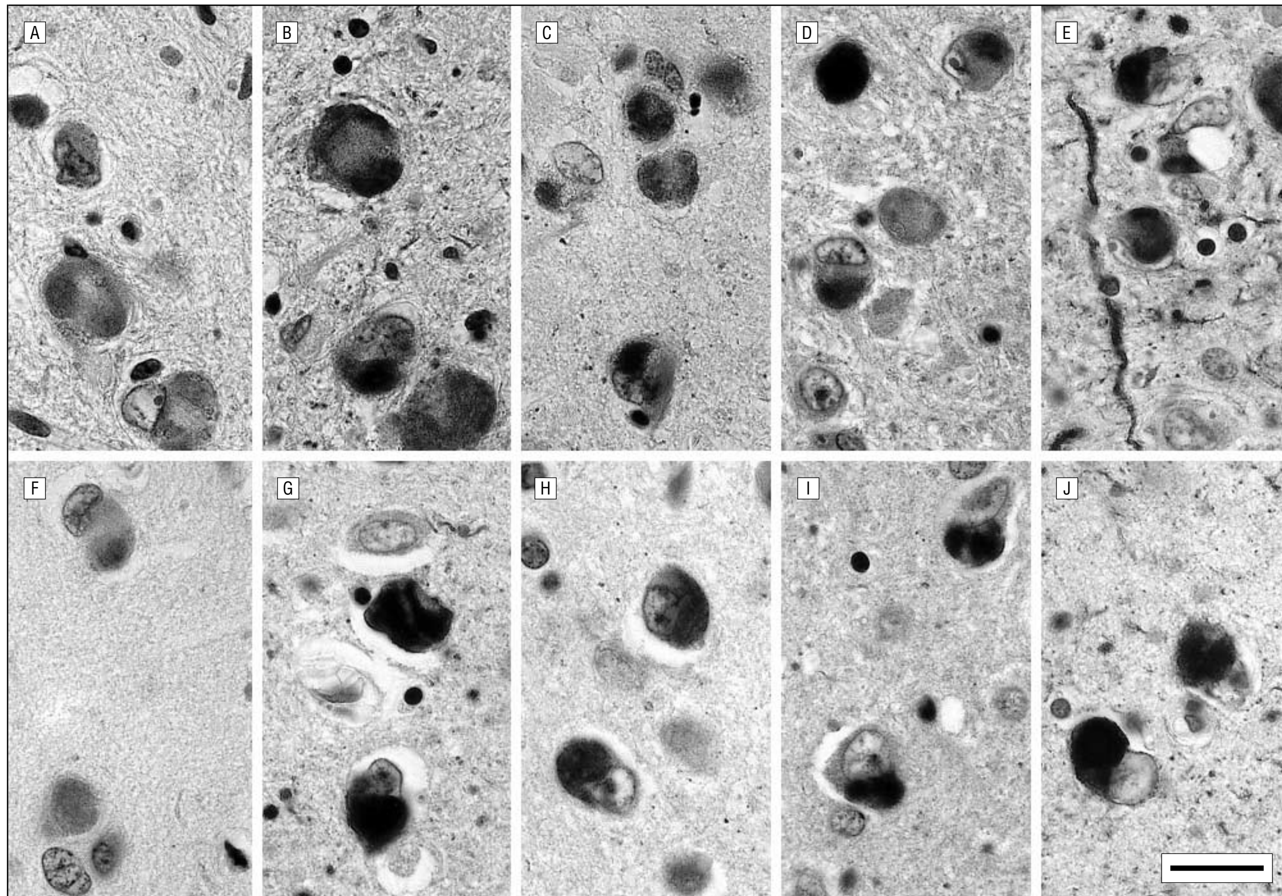

Figure 2. Photomicrographs of α-synuclein epitope recognition in Lewy bodies (LBs) in the amygdala comparing patient 2 with familial Alzheimer disease (A-E) with a patient with dementia with LBs (F-J). The panels show immunostaining for the SNL-4 (A and F), SYN204 (B and G), LB509 (C and H), 211 (D and I), and 202 (E and J) antibodies. Numerous LBs are appreciated using all of the antibodies in both conditions. Similar numbers of LBs stain for SNL-4 compared with the other antibodies, although the intensity of immunoreactivity of SNL-4 is reduced in both patients. Scale bar indicates 20 μm for all images.

sons for these differences are unclear. The differences between LBs and GCIs might be related to conformational differences in the aggregated protein in different cell types, leading to differential exposure of the N-terminal epitopes. The similarity between LBs in FAD, DLB, and PD supports the notion that neuronal LB formation occurs through a single mechanism. Our data suggest that in cases of *PS-1* and *APP* mutation, the formation of LBs is also identical. Although we had only 1 case with an *APP* mutation in the present study, these data build on those of previous work documenting the common occurrence of LBs in these kindreds.[2] We argue that filamentous α-synuclein aggregates in neurons are similar, whether or not the primary disease is a synucleinopathy. Thus, LB formation appears to be a common end point of different genetic pathways.

It could be argued that LB formation is not linked to neuronal degeneration in our patients with FAD, but rather that LBs are a nonspecific manifestation of end-stage neuronal degeneration. All of our subjects had severe dementia with advanced AD pathologic features (Braak stage V or VI).[5] Indeed, LBs are not a feature of preclinical AD related to Down syndrome or *PS* mutations.[2,3] However, the consistently high densities of these inclusions indicate that their occurrence was meaningful. In addition, in patient 5, symptoms of DLB, including marked clinical fluctuations, spontaneous parkinsonism, and visual hallucinations, were present. Patient 2 also had early signs of parkinsonism. Although retrospective examination of medical records showed no definite signs of DLB in the other cases, clinical symptoms or signs of amygdaloid dysfunction were not specifically tracked.

Although α-synuclein, ubiquitin, and neurofilaments are all present in LBs, increasing evidence suggests that α-synuclein is the major protein component of LBs, and mutations of the α-synuclein gene have been shown to be pathogenic for familial PD,[6] whereas α-synuclein has been shown to be incorporated into LBs, pale bodies, and Lewy neurites more consistently and at an earlier point than is ubiquitin.[7] α-Synuclein is a presynaptic protein that is abundantly expressed in neurons throughout the brain, and it is thought to play a role in synaptic plasticity. Furthermore, Richter-Landsberg et al[8] recently showed that during development, oligodendroglial cells express α-synuclein, indicating that α-synuclein is also normally present in these glial cells, thereby implicating α-synuclein in glial functions.

It is unclear why limbic regions are vulnerable to LB formation in FAD. Kosaka[9] noted the amygdala's susceptibility to LB formation when he described a DLB variant with LBs restricted to the cerebral cortex and amyg-

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dala. The amygdala also is susceptible to accumulations of LBs in PD[10] and DLB, and Schmidt et al[11] described susceptibility of the amygdala to LB formation in sporadic AD. In that study, they showed that LBs in the amygdala frequently co-occur with tau-rich neurofibrillary tangles. Thus, amygdaloid neurons appear to be susceptible to LB formation in response to a variety of neurodegenerative disease–initiating events. Studies of the amygdala in other neurodegenerative diseases will help determine whether the propensity to LB formation is restricted to synucleinopathies and amyloidopathies, or whether it is a more universal phenomenon in the degenerating brain.

CONCLUSIONS

In the present study, we compared cases in which disease etiology is linked directly to genetic defects that primarily influence β-amyloid plaque or PS metabolism (or processing) with diseases primarily involving α-synuclein. Although there is no known interaction between APP or PS proteins and α-synuclein, the present study shows that full-length α-synuclein is expressed in LBs in cases in which disease due to genetic mutations is unrelated to the α-synuclein gene. Thus, our data suggest that the mechanisms of LB formation in neurons are identical regardless of the biological trigger, including mutations that cause FAD, but the different α-synuclein epitope profile seen in GCIs in MSA indicates that the mechanism of aggregation of these filamentous inclusions may differ in different cell types.

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