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Immunohistochemical and Biochemical Studies Demonstrate a Distinct Profile of α-Synuclein Permutations in Multiple System Atrophy

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Abstract. Although α-synuclein (α-syn) has been implicated as a major component of the abnormal filaments that form glial cytoplasmic inclusions (GCIs) in multiple system atrophy (MSA), it is uncertain if GCIs are homogenous and contain full-length α-syn. Since this has implications for hypotheses about the pathogenesis of GCIs, we used a novel panel of antibodies to defined regions throughout α-syn in immunohistochemical epitope mapping studies of GCIs in MSA brains. Although the immunostaining profile of GCIs with these antibodies was similar for all MSA brains, there were significant differences in the immunoreactivity of the α-syn epitopes detected in GCIs. Notably, carboxy-terminal α-syn epitopes were immunodominant in GCIs, but the entire panel of antibodies immunostained cortical Lewy bodies (LBs) in dementia with LBs brain with similar intensity. While the distribution of α-syn labeled GCIs paralleled that previously reported using silver stains, antibodies to carboxy-terminal α-syn epitopes revealed a previously undescribed burden of GCIs in the MSA hippocampal formation. Finally, Western blots demonstrated detergent insoluble monomeric and high-molecular weight α-syn species in GCI rich MSA cerebellar white matter. Collectively, these data indicate that α-syn is a prominent component of GCIs in MSA, and that GCIs and LBs may result from cell type specific conformational or post-translational permutations in α-syn.

Key Words: Alpha-synuclein; Dementia with Lewy bodies; Glial cytoplasmic inclusions; Lewy bodies; Multiple system atrophy; Parkinson disease; Synucleinopathies.

INTRODUCTION

In 1969, Graham and Oppenheimer introduced the term multiple system atrophy (MSA) to encompass striatonigral degeneration, olivopontocerebellar atrophy, and Shy-Drager syndrome, which they suggested embodied a spectrum of the same neurodegenerative disorder (1). This notion was later supported by the identification of glial cytoplasmic inclusions (GCIs) as signature lesions of MSA (2, 3). Moreover, the recent demonstration that α-synuclein (α-syn) is a major component of the abnormal filaments that form GCIs provides new opportunities for elucidating the pathogenesis of MSA (4–11). However, this finding was surprising since α-syn is a 140 amino acid phylogenetically conserved protein (12) that is highly abundant in neurons, especially at presynaptic terminals, but it is not expressed at detectable levels in normal glia of the mature mammalian brain (13). Further, mutations in the α-syn gene are pathogenic for familial Parkinson disease (PD) in rare kindreds (14–16) but none have been detected in sporadic PD (17–23) or MSA (22).

While the function of α-syn is unknown, it has been speculated to play a role in regulating synaptic activities (13, 24, 25), and evidence that Lewy bodies (LBs) are formed by filaments composed primarily of abnormal α-syn has implicated this synaptic protein in mechanisms of brain degeneration in sporadic PD, dementia with LBs (DLB) and several subtypes of Alzheimer disease (AD), including the LB variant of AD (LBVAD), as well as familial AD (26–32). Although the precise role that α-syn plays in the development of GCIs and LBs is undetermined, the mechanisms leading to the assembly of α-syn into abnormal filaments may differ for LBs and GCIs. To begin to address this question, we used a panel of antibodies specific for defined epitopes that span the length of α-syn (33) in immunohistochemical studies of GCIs in MSA brains and compared this staining to that found in LBs of DLB cortex. Biochemical fractionation followed by Western blot analysis with the same panel of antibodies to α-syn was also used to dissect variations in soluble as well as insoluble α-syn extracted from GCI rich MSA cerebellar white matter compared with that of control brains.

MATERIALS AND METHODS

Tissue Collection and Processing

The harvesting, fixation and further processing of the tissue specimens studied here were conducted as previously described (26, 34). Briefly, tissue blocks were removed at autopsy from regions representing all of the major neuroanatomical structures

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within the brain and brainstem from the MSA cases and the cingulate cortex of the DLB case (Table 1). The diagnostic assessment of all cases was performed in concordance with published guidelines (35–37). Samples were fixed by immersion in 70% ethanol with 150 mM/l NaCl or 10% buffered formalin for 24–36 h. The samples were dehydrated through a series of graded ethanols to xylene at room temperature and infiltrated with paraffin at 60°C according to a previously described schedule (38). The blocks were then cut into multiple, near serial 6-μm sections for immunohistochemical staining.

Antibodies and Immunohistochemistry

Both rabbit polyclonal and mouse monoclonal (Mab) antibodies to epitopes spanning the entire α-syn molecule were generated as previously described (26, 33). The epitopes recognized by these antibodies in the α-syn molecule are schematically illustrated in Figure 1. The relative activity of each primary antibody was determined and standardized by enzyme-linked immunoabsorbent assay using highly purified human recombinant α-syn (39) and serial dilutions of each antibody. Immunohistochemistry was carried out using the avidin-biotin complex (ABC) detection system (Vector Laboratories, Burlingame, CA) and 3,3′-diaminobenzidine (DAB), as described (28, 38), with some modifications. Briefly, sections were deparaffinized and rehydrated, and selected sections were pretreated with 88% formic acid (FA) for 1 or 5 min to enhance antigen detection. Endogenous peroxidases were quenched with 5% hydrogen peroxide in methanol for 30 min and sections were blocked in 0.1 M Tris with 2% donor horse serum (Tris/DHS) for 5 min. All antibodies were diluted in Tris/DHS. Primary antibodies were incubated overnight at 4°C. After washing, sections were sequentially incubated with species-specific, biotinylated secondary antibodies for 1 h and ABC complex for 1 h. Bound antibody complexes were visualized by incubating sections in a solution containing 100 mM Tris, pH 7.6, 0.1% Triton X-100, 1.4 mM DAB, 10 mM imidazole, and 8.8 mM hydrogen peroxide. Negative controls included the substitution of supernatant from unfused SP2/0-Ag14 mouse myeloma cells for the primary antibodies.

Biochemical Fractionation of α-syn and Western Blot Analysis

Samples of frozen deep cerebellar white matter dissected to exclude deep cerebellar nuclei (0.3 g) from the brains of 3 MSA patients, a PD patient, and a normal control were subjected to serial extractions and Western blot analysis (see Fig. 2 for schematic summary). Tissue was homogenized in 2 ml of high salt (HS) buffer (50 mM Tris, pH 7.4, 750 mM NaCl, 10 mM NaF, 5 mM EDTA with protease inhibitors) per gram of tissue and sedimented at 100,000×*g* for 30 min. The resulting pellet was re-extracted, centrifuged, and both supernatants were pooled (supernatant 1 in Fig. 2), while the pellet (pellet 1 in Fig. 2) was further extracted with HS buffer/10% sucrose to float and remove the myelin. This step generated pellet 2 which was extracted with HS buffer/0.5% Triton X-100 (2 ml/g of tissue) to yield supernatant 2 and pellet 3 which was then extracted with SDS-sample buffer (1% SDS, 10% sucrose, 10 mM Tris, pH 6.8, 1 mM EDTA, 40 mM DTT)(0.33 ml/g of tissue) to generate

TABLE 1
Summary of Cases

Case #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age	55	67	60	65	72	43	74	67	52	62	66	57	58	58	83
Sex	M	F	F	F	F	M	M	F	F	F	M	M	M	M	F
Dx	MSA-P	MSA-P	MSA-P	MSA-P	MSA-P	MSA-P	MSA-P	MSA-P	MSA-P	MSA-P	MSA-P	MSA-C	DLB	PD	NL
Duration	2	3	9	5	14	4	4	8	n/a	3	n/a	n/a	n/a	n/a	n/a
PMI	7	5	11	17	14	16	16	n/a	n/a	n/a	n/a	4.5	12	22	22
Fixative	E	E	E	E	E	E	E	E	F	F	F	F	E	E	E

Summary of the subjects studied here. Each case is numbered 1–15 in the top row and the subsequent rows provide additional information on each of these cases. Abbreviations: Diagnosis (Dx), Parkinsonian-predominant multiple system atrophy (MSA-P), cerebellar-predominant multiple system atrophy (MSA-C), dementia with Lewy bodies (DLB), neurologically normal (NL), postmortem interval (PMI), not available (n/a), ethanol (E), formalin (F).

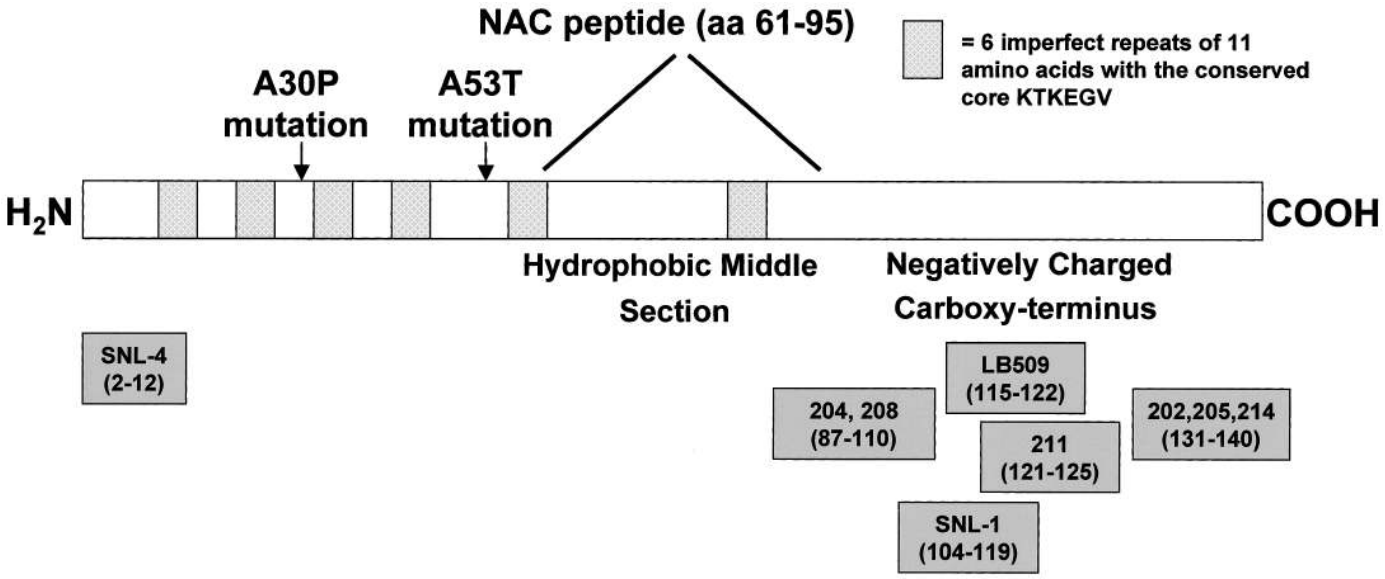

Fig. 1. Synopsis of epitope specificity of α-syn antibody panel. This schematic of human α-syn is not drawn to scale, but illustrates the approximate topography of the 6 imperfect repeats, the non-Aβ component of amyloid plaques (NAC) and the 2 missense mutations that are pathogenic for familial PD. The names of the antibodies used here, together with the amino acid sequences within which their epitopes are located, are indicated below the schematic.

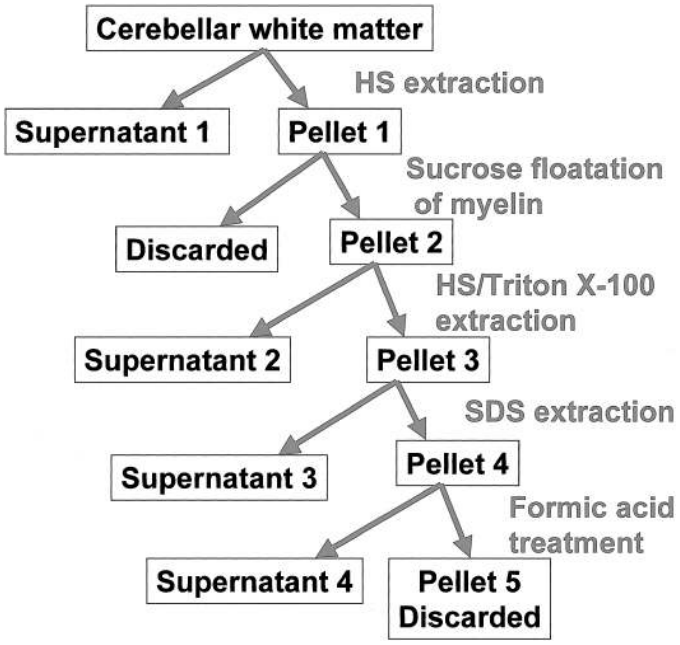

Fig. 2. Protocol for serial extraction of α-syn from cerebellar white matter. To characterize the solubility profile of α-syn in MSA we performed serial extractions, as depicted in the flow diagram, followed by Western blot analysis. See ''Materials and Methods'' for detailed procedure.

supernatant 3 and pellet 4. The SDS-insoluble fraction was extracted with 0.1 ml of 70% FA and sonicated for 2–3 s. FA was lyophilized and the dehydrated material was resuspended in 0.1 ml of SDS-sample buffer and boiled for 10 min. Following centrifugation at 13,000×*g* for 10 min, the pH of the supernatant (supernatant 4) was adjusted with NaOH, and the pellet (pellet 5) was discarded. An equal volume of supernatants 1–4 were then loaded on SDS-polyacrylamide gels, electrophoresed, and the proteins were transferred to nitrocellulose membranes that were probed with α-syn epitope specific antibodies described above and in Figure 1.

Evaluation of Immunohistochemical Data

The distribution and abundance of α-syn-positive GCIs were assessed in each MSA brain using the Syn 202 antibody because it detected these lesions most robustly. The regions of MSA brains that were studied for this purpose included cerebral cortex from the motor, prefrontal, orbital frontal, sensory, Wernicke's, superior temporal, visual, angular and cingulate areas in addition to the hippocampal formation, amygdala, basal ganglia, midbrain, pons, medulla, cerebellum, and thalamus. A semiquantitative assessment strategy was used to estimate the regional density of α-syn immunoreactive inclusions by grading the abundance of these lesions in each region as follows: 3 = frequent, 2 = moderate, 1 = sparse, and 0 = few to none. In cortex, grey and white matter were combined for grading the density of the α-syn positive inclusions and in subcortical and brainstem sites multiple neuroanatomical structures within sections of these regions were pooled together for grading and analysis (Table 2). In the white matter, GCIs were recognized as α-syn positive profiles that occupied most of or nearly the entire cytoplasmic compartment of a cell with the morphology of an oligodendrocyte. In the grey matter, it was not always possible to distinguish α-syn positive GCIs from α-syn positive intra-neuronal inclusions known as neuronal cytoplasmic inclusions (NCIs). Thus, both of these inclusions contribute to the estimates of GCI density in grey matter regions.

To compare the relative immunoreactivity of different α-syn epitopes in LBs and GCIs detected by each antibody used here, serial sections from the cerebellum of 5 MSA cases and the

TABLE 2
Structures in the Brain and Brainstem Assessed for GCI Density

CNS area in Figure 3	CNS structures
Hippocampal Formation	entorhinal cortex
	subiculum
	hippocampal molecular, pyramidal and polymorphic layers
	dentate molecular, granular and polymorphic layers
	alveus
Anterior Basal Ganglia	caudate
	grey matter and pencil fibers of putamen
	nucleus accumbens
	internal capsule
	external capsule
Posterior Basal Ganglia	caudate
	grey matter and pencil fibers of putamen
	globus pallidus interna
	globus pallidus externa
	internal capsule
	external capsule
	anterior commissure
Midbrain	cerebral peduncle
	substantia nigra pars compacta and reticulata
	red nucleus
	medial lemniscus
	brachium of the inferior colliculus
	superior colliculus
	third nerve nucleus
	third nerve
	medial longitudinal fasciculus
Pons	pontine nuclei
	corticospinal tract
	pontocerebellar fibers
	middle cerebellar peduncle
Medulla	corticospinal tract
	olivary nucleus
	olivocerebellar fibers
Cerebellum	cortical Purkinjie, granular and molecular layers
	cortical white matter
	deep cerebellar white matter

cingulate gyrus of the DLB case were probed in the same immunohistochemical experiment by each antibody with and without FA pretreatment. The relative immunoreactivity of these epitope specific antibodies for LBs and GCIs was assessed using the following semiquantitative strategy: 3 = robust immunostaining; 2 = moderate immunostaining; 1 = faint immunostaining; 0 = no immunostaining.

RESULTS

Distribution of α-syn-positive GCIs in Different Regions of MSA Brains

Using Syn 202, a Mab that recognizes an epitope in the last 10 amino acids of α-syn (i.e. amino acids 130–140), the regional abundance and distribution of α-syn immunoreactive GCIs were examined in 12 MSA cases. Figure 3 summarizes the GCI density in the different neuroanatomical structures studied as a percentage of the total number of cases from whence each area was available.

Numerous neuroanatomical regions consistently contained a high density of GCIs including the posterior basal ganglia, pons, cerebellum and medulla, which is consistent with previous descriptions of GCI density conducted with silver staining techniques. Similarly, in accordance with previous assessments, numerous regions were consistently characterized by low GCI density including the non-motor cortical white matter (e.g. superior temporal gyrus, visual cortex, Wernicke's area), and thalamus. Of note, the hippocampal formation was found to have high to moderate GCI density in 25% of the cases examined here (Fig. 3). In these cases with abundant GCI density, numerous areas within the hippocampal formation contained GCIs including the entorhinal and subicular white matter, stratum oriens, stratum radiatum, and alveus, as well as abundant neuronal inclusions in the stratum granulosum of the dentate gyrus consistent with previously described inclusions termed UNIDs by Takeda et al (40), and neuronal cytoplasmic staining in the stratum pyramidale of the CA subfields (Fig. 4). Interestingly, these cases with high GCI and NCI density in the hippocampal formation also exhibited apparent presynaptic axonal terminal pathology in the hilum (panel D, Fig. 4) similar to what has been previously described in PD and DLB by Galvin et al (41).

Detection of Topographically Distinct α-syn Epitopes in GCIs

For comparison of the α-syn epitopes detected in GCIs with those detected in LBs of the DLB brain, we examined sections of MSA and DLB brains. Similar to previously reported immunostaining in nigral LBs (33), all of the α-syn antibodies stained cortical LBs without FA pretreatment (Fig. 5). In contrast, only those antibodies specific for epitopes between amino acids 130–140 (Syn 202, Syn 205, Syn 214) in the C-terminus of α-syn yielded similar robust staining of GCIs without FA pretreatment of the MSA brain sections (Fig. 6). Moreover, the immunostaining of GCIs with the antibodies specific for the other α-syn epitopes was less robust and more variable in closely adjacent sections of the same MSA brains (Fig. 7). For example, without FA pretreatment, the α-syn immunoreactivity of GCIs was faint with the SNL-4 and LB509 antibodies, and virtually absent with the Syn 204, Syn 208, SNL-1, and Syn 211 antibodies (Fig. 7, top row). Similar results were obtained in the 5 different cases of MSA studied in this manner (Fig. 8). However, after 1 min of FA pretreatment, the GCI α-syn immunostaining improved variably for some but not all antibodies (Fig. 7, bottom row), and 5 min FA pretreatment

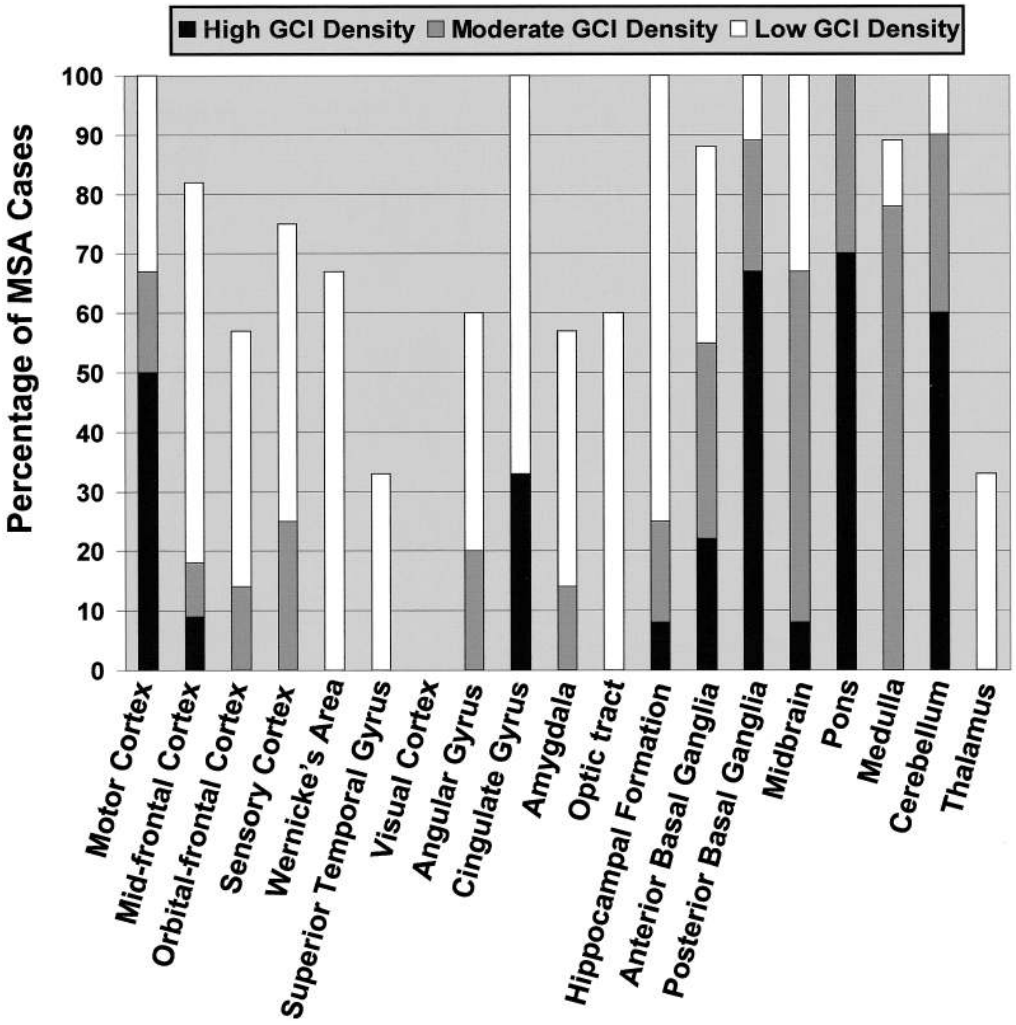

Fig. 3. Regional abundance of GCIs/NCIs in MSA brains. The number of cytoplasmic inclusions was assessed semiquantitatively for multiple neuroanatomical structures and pooled into groups as outlined in Table 2. The GCI density of each anatomical area is represented as a percentage of the total number of cases for each degree of density.

increased immunostaining of GCIs with all α-syn antibodies specific for epitopes outside the C-terminus of α-syn (data not shown).

Biochemical and Western Blot Analysis

As shown in Figure 9, the cerebellar white matter from 3 MSA brains, 1 PD case, and 1 neuropathologically normal brain were studied by biochemical fractionation followed by Western blot analysis using anti-α-syn antibodies LB509, Syn 208, SNL-1, Syn 2ll and Syn 204 and anti-α/β-syn antibody Syn 205 (6,33). In the HS-soluble fractions (see the first of the 3 panels shown in Fig. 9), a ~18 kD immunoband corresponding to α-syn was detected in the MSA, PD, and control brains with each of the epitope-specific antibodies to α-syn. β-Syn was detected in HS-soluble extracts with Syn 205 Mab and the levels of both α- and β-syn were reduced in the HS-soluble fraction of MSA brains (Fig. 9A). Both α- and β-syn were detected at very low levels in the HS/0.5% Triton X-100 fractions (data not shown). In the SDS-soluble and FA-soluble fractions α-syn but not β-syn immunobands were present in all of the MSA brains, but not in the PD and control brains (see last 2 panels in Fig. 9). Finally we detected higher molecular weight α-syn aggregates only in these 2 fractions of the MSA brains (e.g. see bands marked by an arrow in Fig. 9A–C), which may correspond to α-syn dimers for reasons summarized in the discussion.

DISCUSSION

In this study, we expanded our previous investigations (6) into the alterations of α-syn that occur in GCIs of MSA brains. It is now well recognized that α-syn immunostaining is more sensitive than either silver staining or ubiquitin immunostaining for detecting GCIs in sections of MSA brains (4–9). In order to determine whether

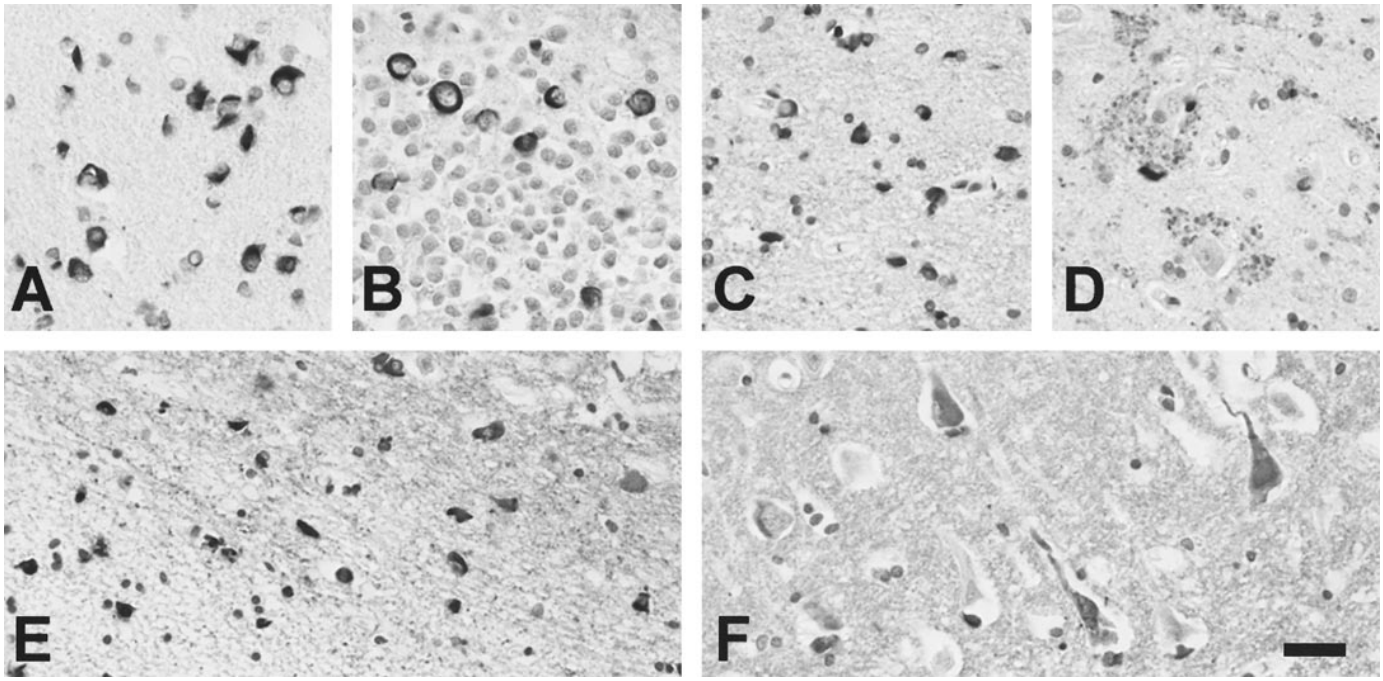

Fig. 4. α-Syn pathology in the hippocampal formation of MSA. Immunostaining with Syn 202 in the subicular white matter (A), stratum granulosum of the dentate gyrus (B), stratum radiatum (C), hilum (D), stratum pyramidale, stratum oriens and alveus (E), and stratum pyramidale of CA1 (F) in a case of MSA. Note the abundant GCIs in (A), (C) and (E), the neuronal inclusions in (B) and (F) and the axonal terminal pathology in (D). Scale bar = 30 μm.

GCIs were more pervasive and/or abundant than reported in earlier studies using silver staining methods (42–44), or ubiquitin immunostaining (45), we examined 12 MSA brains by immunohistochemistry with the α-syn specific antibody, Syn 202. Areas that consistently have a high GCI density include the motor cortex, posterior basal ganglia, pons, and cerebellum. Significantly, one area that appears to distinguish α-syn immunolabeled from silver-stained GCIs in the MSA brain is the hippocampal formation, since it was reported that this region was ''free or nearly free'' of silver-positive GCIs (42), while α-syn specific antibodies detected a high to moderate GCI density in the hippocampal formation of 25% of MSA cases, though they all had at least some hippocampal formation GCIs (Fig. 3). It would be interesting to correlate the GCI/NCI burden in the hippocampal formation with clinical assessments of cognitive dysfunction that occasionally occurs in MSA (46). In contrast, other areas that were found to be ''free or nearly free'' of GCIs with silver staining (e.g. parietal sensory, occipital visual and temporal cortex as well as the thalamus) had very few GCIs by α-syn immunostaining suggesting that the sensitivity of antibodies to α-syn for detecting GCIs is not increased dramatically over silver staining methods throughout the MSA brain.

In a recent immunohistochemical study with antibodies specific to a myelin basic protein epitope selectively exposed in damaged myelin, Matsuo et al demonstrated widespread myelin degeneration in more MSA brain regions than had been described previously with less sensitive myelin staining techniques (47). Moreover, based on data from studies using anti-ubiquitin antibodies and immunohistochemistry, the authors suggested that demyelination occurs in areas without evidence of GCI formation or neuronal loss. However, it is conceivable that α-syn immunostaining might reveal more GCIs in these areas such that a correlation between GCI distribution and demyelination could be demonstrated using antibodies to α-syn and damaged myelin. In contrast, Papp and Lantos found that GCIs were present in areas lacking neuronal loss, gliosis, or myelin pallor, suggesting that GCI formation may precede these other neuropathological abnormalities (42). The distribution of α-syn positive GCIs reported herein supports this hypothesis, particularly in the hippocampal formation, which does not normally exhibit the other secondary changes but has α-syn positive GCIs.

The differences in immunostaining of LBs and GCIs reported here is the first evidence to suggest structural differences in the α-syn comprising LBs and GCIs. Given that all of the antibodies to α-syn detected LBs and GCIs, (albeit after vigorous antigen retrieval for GCIs) by immunohistochemistry, the entire α-syn polypeptide is likely present in both of these lesions and it is improbable that their formation occurs secondary to partial degradation of α-syn. It is possible that the differences

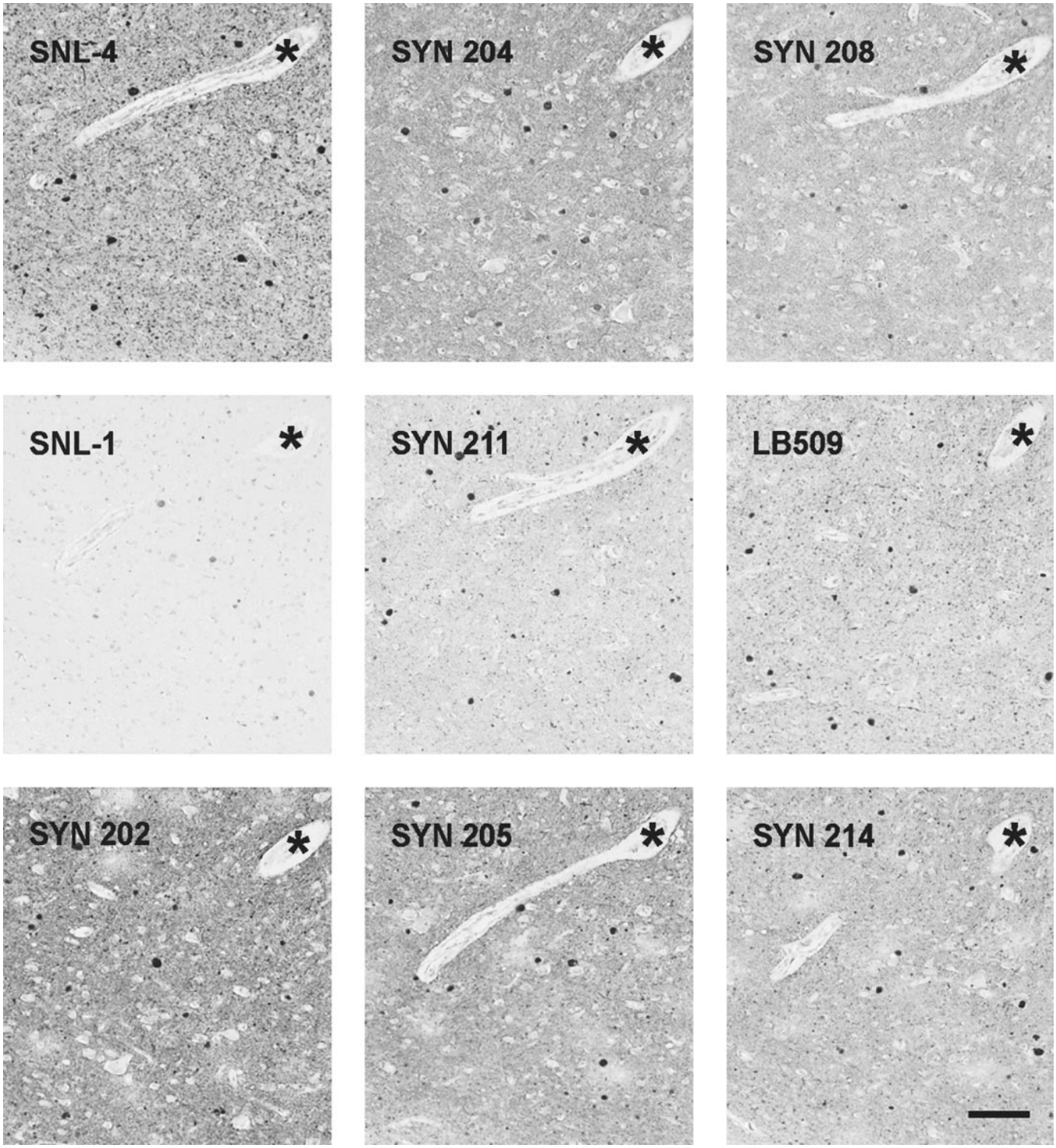

Fig. 5. α-Syn epitope recognition in DLB. Immunostaining for each α-syn antibody in serial sections of the cingulate cortex of 1 case of DLB. α-Syn-positive LBs are clearly immunostained with each antibody. The asterisk marks the same blood vessel in each section. Scale bar = 100 μm.

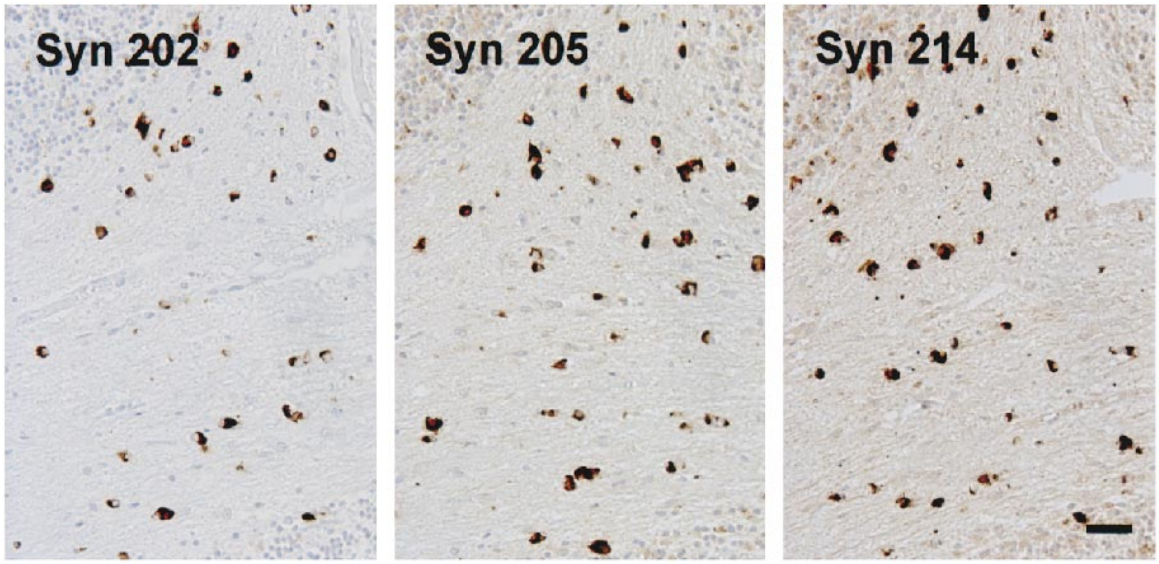

Fig. 6. Carboxy-terminal antibody recognition of GCIs. Immunostaining for each carboxy-terminal α-syn antibody without FA pretreatment in serial sections of cerebellar white matter from 1 case of MSA. Note the robust staining of GCIs with each antibody. Scale bar = 30 μm.

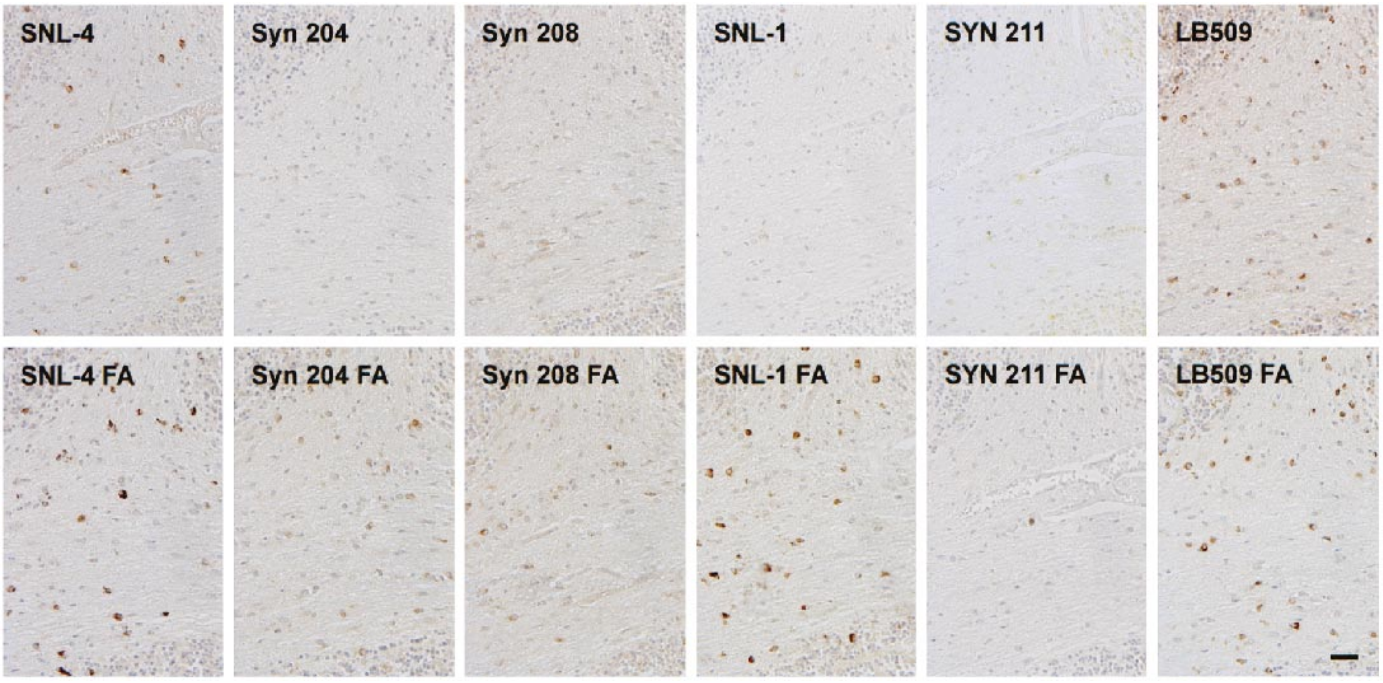

Fig. 7. Non-carboxy-terminal antibody recognition of GCIs. Immunostaining of GCIs in serial sections of cerebellar white matter from the same case as in Figure 6 with the non-carboxy-terminal antibodies. Antibodies are arranged in an amino- to carboxy-terminal array. Note that without FA pretreatment there is only faint GCI immunostaining with SNL-4 and LB509 and little or no GCI immunostaining with the remainder of antibodies (Top row). With FA pretreatment for 1 min the CGI immunostaining improves variably for each antibody (Bottom row). Scale bar = 30 μm.

in immunostaining are due to different assembly intermediates and/or co-assembly partners, although undetermined post-translational modifications may also be involved. Previous studies reported that there are morphological differences in the ultrastructure of LBs and GCIs (2, 6, 8, 9, 26, 27, 34, 44, 48, 49), as well as variations in the other major proteins within these 2 lesions. The second most common protein detected in LBs are the neurofilament subunits (34, 50, 51), while αB-crystallin, α- and β-tubulin have been found in GCIs

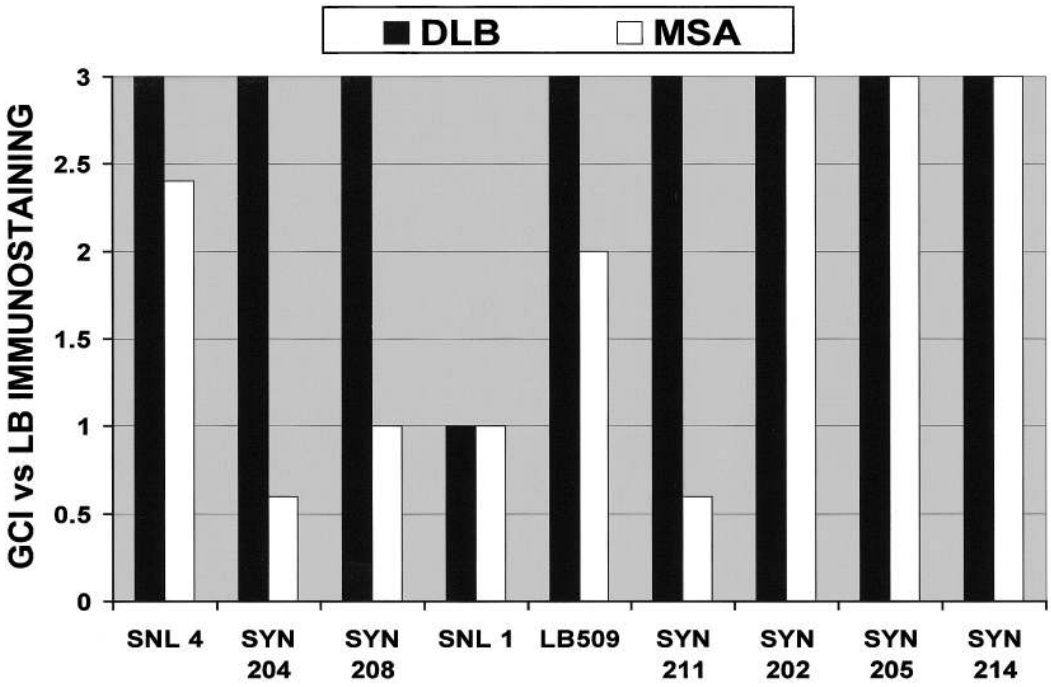

Fig. 8. Summary of α-Syn epitope recognition. α-Syn antibody recognition of the LBs of 1 case of DLB is compared with the recognition of GCIs in 5 different cases of MSA. Immunostaining scale: 3 = robust immunostaining; 2 = moderate immunostaining; 1 = faint immunostaining; 0 = no immunostaining. Value of MSA bars represents an average of the 5 cases. Antibodies are displayed in an amino- to carboxy-terminal array. Note the similar robust staining with the 3 carboxy-terminal antibodies within each disorder and the decreased immunostaining of GCIs with the remainder of antibodies.

(49). It is conceivable that interactions of α-syn with 1 or more of these potential co-assembly proteins underlie the variability in α-syn epitope recognition observed here. Further, differences in the cytoplasmic milieu of oligodendrocytes and neurons may predispose to different ultrastructures. For example, the cytoplasmic domain of oligodendrocytes is devoid of intermediate filaments, and many other differences, including the metabolic activities involved in myelin generation, may contribute to the altered structure of the aggregated proteins as well as to the selective vulnerability of different cells to the accumulation of filamentous α-syn inclusions. Future investigations may clarify this issue and lead to a better understanding of the role that α-syn plays in the development of these inclusions.

The studies here of α-syn following biochemical fractionation of cerebellar white matter demonstrated reduced α-syn levels in the HS-soluble fraction concomitant with an increase in the SDS- and FA-extractable fractions in all 3 MSA brains examined. Thus, it is tempting to interpret these results to reflect a redistribution of this protein from one pool to another in MSA. However, it is likely that both changes are due to 2 processes occurring in 2 different cell types. Since α-Syn is a synaptic protein that is not detected in normal oligodendrocytes, it is likely that most of the HS-soluble α-syn represents protein in axons being transported to the axonal termini. The reduction in HS-soluble α-syn in MSA cerebellar white matter may reflect a reduction in axonal transport. This is consistent with a similar reduction in β-syn in the HS-soluble, but without an accumulation in the SDS- or FA-extracted fractions. The presence of α-syn in the SDS- and FA-extracted fractions of MSA cerebellar white matter, but not in control tissue, is consistent with the notion that these altered forms of α-syn are a reflection of the GCIs in this region of the MSA brain. A recent study by Dickson et al (4), using similar sequential extraction procedures, demonstrated widespread alterations in the solubility of α-syn (as reflected by an increase of α-syn in SDS- and FA-soluble fractions) that did not correlate with the densities of GCIs. Whereas Dickson et al utilized brain samples that combined both gray and white matter, our Western blot results reflect only α-syn in the white matter of MSA brains. The observation by Dickson et al suggests that alterations in the solubility of α-syn may occur independently of GCI formation in MSA brains and that some alterations may occur within cell populations other than oligodendrocytes. Although we did not systematically attempt to correlate the GCI density with changes in the solubility of α-syn, we only detected SDS- and FA-extractable α-syn in the cerebellar white matter of MSA brain and not in control brain,

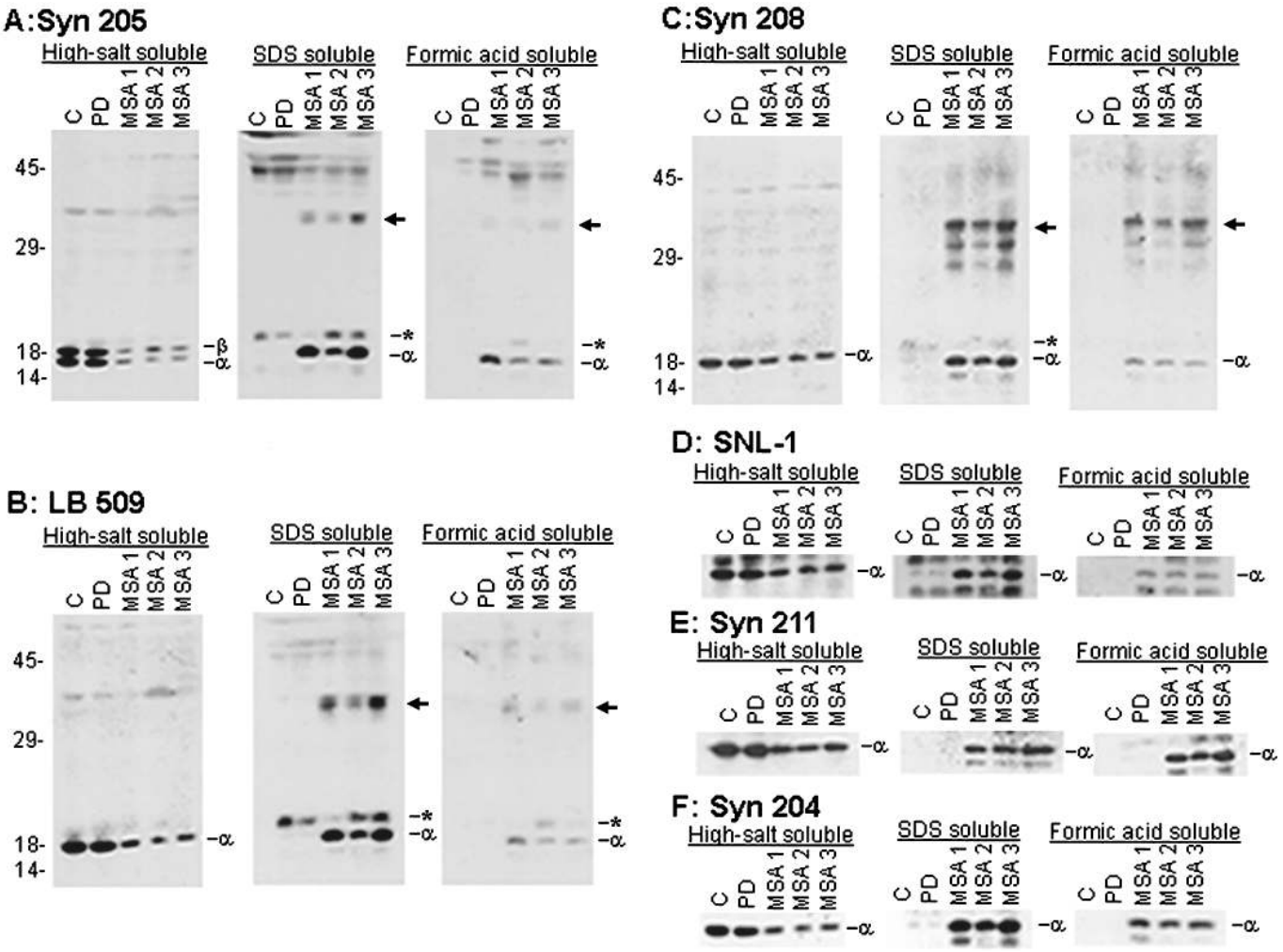

Fig. 9. Biochemical fractionation and Western blot analysis of cerebellar white matter from MSA and control cases. Cerebellar white matter from 3 MSA patients (MSA1–3), a Parkinson disease patient (PD) and a neuropathologically normal control patient (C) was dissected and fractionated as outlined in Figure 2. Equal volume of each fraction was loaded on 12% polyacrylamide gels and transferred to nitrocellulose membranes, which were probed with the various α-syn antibodies indicated. α-Syn, but not β-syn, is present in the SDS- and FA-soluble fractions. The band indicated by the asterisk is a cross-reactive band not specific to the primary antibodies. Note also the presence of higher molecular mass α-syn aggregates (arrows) in these fractions. The molecular masses (kD) of markers are depicted to the left of the blots.

which strongly suggests that these altered forms of α-syn do indeed reflect the abundance of GCIs.

Western blot analysis of MSA brain also revealed higher-molecular weight species (~36 kDa) of α-syn specifically in the SDS- and FA-fractions, also suggesting that this modified form of α-syn is a component of GCIs. Since these proteins migrate at approximately twice the normal molecular mass of full-length α-syn, they may represent dimeric covalently cross-linked protein. Significantly, small complexes of α-syn can promote the elongation of filaments in vitro (52). Thus, further characterization of these higher molecular weight species of α-syn in the MSA brain may provide insight into the initial steps leading to the formation of GCIs (39). Since this research could reveal novel targets for the focused design of new drugs to block or retard the progressive accumulation of GCIs in MSA brain, we speculate that 1 or more of these drugs might have therapeutic value for the treatment of MSA and, possibly, other synucleinopathies.

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