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Analysis and comparison of post-translational modifications of α-synuclein filaments in multiple system atrophy and dementia with Lewy bodies

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Our previous cryogenic electron microscopy (cryoEM) analysis showed that the core structures of α-synuclein filaments accumulated in brains of patients diagnosed with dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) patients are different. We analyzed the post-translational modifications (PTMs) in these filaments , and examined their relationship with the core filament structures and pathological features. Besides the common PTMs in MSA and DLB filaments, acetylation, methylation, oxidation and phosphorylation were frequently detected in MSA filaments, but not in DLB filaments. Furthermore, in DLB filament cases, the processing occurred at the C-terminal side of Asp at 119 residue and Asn at 122 residue, while in MSA cases, the processing occurred at multiple sites between residues 109–123. We have previously reported that PTMs in tau filaments depend on the filament core structure. This was considered to apply to α-synuclein filaments as well. As an example, PTMs including processing sites detected in α-synuclein filaments in early-onset DLB (an atypical form, now named juvenile-onset α-synucleinopathy) brain also supported this idea. These suggests that PTMs appeared to be closely related to the specific filament core structures.

Keywords α-Synuclein, Multiple system atrophy, Dementia with Lewy bodies, Cryogenic electron microscopy, Mass spectrometry, Post-translational modification

α-Synuclein consists of 140 amino acid residues. The N-terminus (residues 1–60) contains KTK lipid-binding motif repeats associated with vesicle binding[1–4]. The central region (residues 61–95) is the non-amyloid-ß component (NAC) region[5], which is essential for α-synuclein aggregation[6,7]. The highly unstructured C-terminal region (residues 96–140) can bind calcium and is populated by negatively charged residues[8–10]. Calcium binds to the C-terminus of α-synuclein, thereby increasing its lipid-binding capacity, and this phenomenon may be involved in synaptic vesicle homeostasis[11].

α-Synucleinopathy is characterized by accumulation of misfolded α-synuclein aggregates. α-Synuclein is the major component of the filamentous inclusions found in neuronal and/or glial cells in several neurodegenerative diseases[12]. In Parkinson's disease (PD) and dementia with Lewy bodies (DLB), α-synuclein pathologies are mainly observed in neurons and neurites in the form of Lewy bodies (LBs) and Lewy neurites (LNs)[13,14], whereas glial cytoplasmic inclusions (GCIs) are seen in oligodendrocytes in multiple system atrophy (MSA)[15]. A causal relationship between α-synuclein aggregates and disease was established by the findings that missense mutations in *SNCA* (the gene that encodes α-synuclein) and multiplications of this gene give rise to rare inherited forms of PD and PD with dementia[16–20]. To date, various missense mutations in the SNCA gene, p.A53T, p.A18T,

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p.A29S, p.A30P, p.E46K, p.G46K, p.H50Q, p.G51D, and p.A53E, have been identified in familial forms of PD and DLB[16,21–30]. These pathogenic mutations affect alpha-synuclein fibril formation in vitro, either accelerating fibril formation[31–34] or resulting in the formation of fibrils that are more fragile and easier to propagate than wild-type (WT) fibrils[35]. These abnormal α-synuclein fibrils are accumulated as fibrous forms[36,37], existing in phosphorylated and partially ubiquitinated states[38,39], and exhibit seeding activity to induce prion-like conversion, detergent-insolubility and protease resistance compared to endogenous α-synuclein[40].

We have established by means of cryogenic electron microscopy (cryoEM) analysis that the core structures of α-synuclein filaments accumulated in the brains of PD/PDD/DLB and MSA patients are different[41,42].

In this study, we analyzed the relationships among filament structures, post-translational modifications (PTMs), and characteristic pathological structures in α-synucleinopathies. Our results suggest that the core structure of α-synuclein filaments and PTMs are not important factors for the formation of Lewy bodies and Lewy neurites.

Results and discussion

As shown in Fig. 1 and supplementary information, ubiquitination of residues 6 K, 12 K, 21 K, and 23 K, acetylation of 1Met, deamination of 62Q, 65N, 103N, 109Q, and 122N, dimethylation of 80 K, trimethylation of 60 K, and phosphorylation of 129S, and 136Y (or 133Y) were commonly detected in sarkosyl-insoluble α-synuclein from MSA and DLB patients' brains, with a few exceptions.

In addition, acetylation of residues 12 K, 23 K, 32 K, 34 K, 45 K, 58 K, 60 K, 80 K, 97 K, and 102 K, methylation of 12 K, 60 K, 80 K, and 96 K, oxidation of 1 M, 5 M, 50H, 116 M, and 127 M, and phosphorylation of 39Y, 59 T, 64 T, and 92 T were found in MSA patients' brains.

However, these MSA PTMs were not detected in in sarkosyl-insoluble α-synuclein from DLB patients' brains. Furthermore, acetylation of Lys residues and Met residues in the N-terminal region 1–40 of α-synuclein from MSA was clearly increased compared to that of alpha-synuclein from DLB.

There have already been several reports on post-translational modifications of α-synuclein[43–45], but all of them analyzed α-synuclein in the soluble fraction of the brain or the mixture of soluble and insoluble fractions. Furthermore, in those reports, the detected post-translational modifications were summarized by disease, so comparison between individuals as in this study is not possible. Therefore, it cannot be directly compared with this study, which analyzed α-synuclein in the insoluble fraction (filament α-synuclein) on an individual basis, but the results are listed in Supplemental Table 4. In addition, α-synuclein in normal brains were also analyzed, and the results are also listed in Supplemental Table 4.

In normal brains, phosphorylation, ubiquitination, and acetylation could not be detected. In previously reported normal brains, ubiquitination was not detected, but acetylation and phosphorylation were detected as shown in Supplemental Table 4. The normal brain samples previously reported included brains with Primary age-related tauopathy[43], and were not completely normal, so we believe that this is the reason for the difference.

In the soluble fractions of MSA and DLB brains, disease-related differences in PTMs were observed only in some phosphorylations. In this study, disease-related differences in some phosphorylations were observed in the insoluble fractions as well as in the soluble fractions (Supplemental Table 4). Regarding acetylation, differences were observed between the insoluble and soluble fractions, and further investigation is necessary.

We previously showed by cryoEM analysis that the core structures of α-synuclein filaments accumulated in the brains of PD/PDD/DLB and MSA patients are different. The filament core region of α-synuclein involves approximately residues 14–99 in MSA and residues 31–100 in PD/DLB, while other areas are considered to form the fuzzy coat region[41,42]. The differences in PTMs in the core region between DLB and MSA, especially the acetylation level of Lys residues, may be significant.

There was less synuclein accumulation in DLB, and we cannot rule out the possibility that some PTMs were not detected due to the small amount of α-synuclein recovered from the sarcosyl-insoluble fraction of DLB brains.

Recently, a new α-synuclein fold has been reported that differs from the folds seen in LBD and MSA[46]. In JOS, a 21-nucleotide duplication in one allele of SNCA was previously identified in a patient with abundant α-synuclein inclusions[46,47]. Severe neuronal loss and gliosis in the cerebral cortex and substantia nigra were present alongside vacuolar changes in the upper layers of the neocortex. α-Synuclein pathology exceeded that of typical DLB[46,47]. The cryo-EM structures of α-synuclein filaments from JOS brain are different from those of the Lewy fold observed in PD and DLB, and share only a partial similarity with the structures of MSA filaments, as shown in Fig. 2 (in the area surrounded by red lines)[46].

In α-synuclein filaments from JOS, acetylation of residues 34 K, 45 K, 58 K, and 60 K, methylation of 43 K, 58 K, and 96 K, oxidation of 1 M and 50H, and phosphorylation of 33 T and 39Y were detected, in addition to PTMs that are relatively common in MSA and PD/DLB. The PTMs pattern of JOS was similar to that of MSA, but very different from that of DLB (Fig. 1A,B).

Furthermore, it is known that processing occurs at the C-terminus of synuclein[48–50]. In this study, mass spectrometry analysis of α-synuclein filaments in the sarkosyl-insoluble fraction identified multiple peptide fragments corresponding to the C-terminal cleavage site. The cleavage sites revealed from the results are shown in Table 1 and Fig. 3. In DLB cases, the processing occurred at the C-terminal side of Asp at 119 residue and Asn at 122 residue, while in MSA cases and the JOS case, the processing occurred at multiple sites between residues 109–123 and 121–130 (114–123 MSA numbering) regions, respectively. These C-terminal processing sites of MSA and JOS are similar, but again are different from that of DLB, as shown in Fig. 3 and Table 1. These processing enzyme may be involved in filament formation, so further elucidation is needed.

We have previously reported that PTMs in tau filaments depend on the filament core structure[51]. This was considered to apply to synuclein filaments as well. In JOS, the core structure is formed of filaments that are different from ordinary DLB filaments, and rather similar to MSA filaments. Furthermore, in terms of PTMs,

A

Amino acid residue and modification	MSA-1	MSA-2	MSA-3	MSA-4	MSA-5	DLB-1	DLB-2	DLB-3	DLB-4	DLB-5
33T, phosphorylation	0 / 3	0 / 5	0 / 5	0 / 3	0 / 4	0 / 4	-	-	-	-
39Y, phosphorylation	0 / 12	1 / 16	0 / 12	0 / 18	1 / 35	0 / 13	0 / 9	0 / 13	0 / 9	0 / 5
59T, phosphorylation	0 / 5	0 / 9	1 / 4	0 / 6	1 / 18	0 / 10	0 / 5	0 / 4	0 / 1	-
64T, phosphorylation	0 / 191	0 / 135	1 / 170	0 / 186	2 / 226	0 / 79	0 / 24	0 / 35	0 / 51	0 / 20
92T, phosphorylation	0 / 108	0 / 105	0 / 96	0 / 132	1 / 159	0 /85	0 / 16	0 / 21	0 / 21	0 / 11
129S,	2 / 6	1 / 9	0 / 5	2 / 12	2 / 11	8 / 31	1 / 7	3 / 11	2 / 8	0 / 3
136Y, phosphorylation	1 / 6	1 / 9	1 / 5	2 / 12	2 /11	1 / 31	1 / 7	1 / 11	0 / 8	0 / 3
6K, ubiqutination	1 / 1	2 / 3	2 / 2	2 / 3	1 / 7	3 / 4	1 / 1	2 / 2	1 / 1	-
12K, ubiqutination	2 / 3	3 / 4	1 / 3	2 / 4	1 / 4	1 / 6	1 / 1	2 / 2	0 / 1	1 / 1
21K, ubiquitination	0 / 7	2 / 8	0 / 9	2 / 11	1 / 25	2 / 13	1 / 9	3 / 15	0 / 21	1 / 3
23K, ubiquitination	1 / 3	0 / 3	1 / 3	1 / 4	1 / 5	2 / 5	2 / 4	2 / 7	1 / 3	1 / 4
45K, ubiquitination	-	0 / 2	-	0 / 3	1 / 3	0 / 3	0 / 1	-	-	-
60K, ubiquitination	0 / 5	1 / 9	0 / 4	0 / 6	0 / 18	0 / 10	4 / 5	2 / 4	1 / 3	-
80K, ubiquitination	0 / 191	0 / 135	0 / 170	0 / 196	2 / 226	0 / 79	0 / 24	0 / 35	0 / 55	0 / 20
102K, ubiquitination	0 / 22	0 / 29	0 / 25	0 / 38	0 / 55	0 / 31	0 / 6	0 / 16	0 / 11	0 / 4
N-terminal acetylation	1 / 1	3 / 3	2 / 2	3 / 3	6 / 7	4 /4	1 / 1	2 / 2	1 / 1	-
12K, acetylation	1 / 3	0 / 4	1 / 3	0 / 4	0 / 4	0 / 6	0 / 1	0 / 2	0 / 1	0 / 1
23K, acetylation	1 / 3	0 / 3	1 / 3	1 / 4	1 / 5	0 / 5	0 / 4	0 / 7	0 / 3	0 / 4
32K, acetylation	0 / 2	0 / 5	0 / 10	0 / 5	1 / 9	0 / 6	0 / 4	0 / 5	0 / 2	0 / 3
34K, acetylation	0 / 3	1 / 5	1 /5	1 / 3	1 / 13	0 / 4	-	-	-	-
45K, acetylation	-	0 / 2	-	0 / 2	1 / 3	0 / 3	0 / 1	-	-	-
58K, acetylation	0 / 24	1 / 27	0 / 23	1 / 72	2 /85	0 / 31	0 / 16	0 / 19	0 / 12	0 / 7
60K, acetylation	0 / 5	3 / 9	0 / 4	2 / 6	2 / 18	0 / 10	0 / 5	0 / 4	0 / 3	-
80K, acetylation	0 / 191	2 / 135	0 / 170	2 / 196	4 / 226	0 / 79	0 / 24	0 / 35	0 / 55	0 / 20
97K, acetylation	0 / 23	1 / 29	0 / 25	0 / 39	0 / 57	0 / 6	0 / 6	0 / 10	0 / 8	0 / 3
102K, acetylation	0 / 22	0 / 29	0 / 25	0 / 38	0 / 55	0 / 31	0 / 6	0 / 16	0 / 11	0 / 4
12K, methylation	0 / 3	0 / 4	0 / 3	0 / 3	1 / 4	0 / 6	0 / 1	0 / 2	0 / 1	0 / 1
58K, methylation	0 / 24	0 / 27	0 / 23	0 / 72	0 / 85	0 / 31	0 / 16	0 / 19	0 / 12	0 / 7
60K, metylation	0 / 5	0 / 10	1 / 5	0 / 5	1 / 11	0 / 10	0 / 5	0 / 4	0 / 3	-
80K, methylation	0 / 191	0 / 135	0 / 170	0 / 196	5 / 226	0 / 79	0 / 24	0 / 35	0 / 55	0 / 20
96K, methylation	0 / 108	0 / 114	0 / 98	0 / 131	1 / 166	0 / 85	0 / 16	0 / 21	0 / 20	0 / 11
45K, dimethylation	0 / 1	1 / 3	-	1 / 5	0 / 3	0 / 3	-	0 / 1	0 / 1	-
58K, dimethylation	1 / 25	0 / 27	0 / 23	0 / 72	2 / 80	2 / 31	0 / 16	1 / 19	1 / 12	0 / 7
60K, dimethylation	2 / 7	2 / 11	0 / 4	2 / 6	9 / 23	1 / 10	0 / 5	0 / 4	1 / 3	-
80K, dimethylation	2 / 194	2 / 135	2 / 170	3 / 196	13 / 226	2 / 79	0 / 24	0 / 35	2 / 55	1 / 20
96K, dimethylation	0 / 110	0 / 107	0 / 98	0 / 131	0 / 166	0 / 85	0 / 16	0 / 22	0 / 21	0 / 10
34K, trimethylation	0 / 3	1 / 5	0 / 5	0 / 3	0 / 13	1 / 4	-	-	-	-
60K, trimethylation	0 / 7	1 / 11	0 / 4	1 / 6	1 / 2	2 / 10	0 / 5	1 / 3	1 / 2	-
80K, trimethylation	1 / 194	1 / 135	0 / 170	0 / 196	0 / 226	1 / 79	0 / 24	0 / 35	0 / 55	0 / 20
24Q, deamination	0 / 3	1 / 5	0 / 5	0 / 3	2 /13	0 / 6	0 / 4	3 / 7	0 / 5	0 / 4
62Q, deamination	1 /191	3 / 135	1 / 170	3 / 196	9 / 226	2 / 79	2 / 24	0 / 35	1 / 55	0 / 20
65N, deamination	0 / 191	1 / 135	3 / 170	1 / 196	19 / 226	3 / 79	4 / 24, 1/1	6 / 35	1 / 55	0 / 20
72T, phosphorylation	0 / 191	0 / 135	0 / 170	0 / 196	0 / 226	0 / 79	0 / 24	0 / 35	0 / 55	0 / 20
79Q, deamination	1 / 191	4 / 135	1 / 169	4 / 196	7 / 226	0 / 79	0 / 24	0 / 35	0 / 55	0 / 20
99Q, deamidation	0 / 22	0 / 29	0 / 25	0 / 38	4 / 54	1 / 31	0 / 6	0 / 16	0 / 11	0 / 4
103N, deamidation	23 / 24	23 / 29	23 / 25	22 / 41	41 / 60	45 / 50	8 / 12	15 / 16	11 / 11	4 / 4
109Q, deamidation	7 / 24	2 / 22	3 / 25	12 / 41	9 / 60	18 / 60	3 / 12	4 / 16	2 / 11	2 / 4
122N, deamidation	3 / 10	5 / 2	1 /10	5 / 18	5 / 20	23 / 34	2 / 7	1 / 13	4 / 10	1 / 3
134Q, deamidation	0 / 6	2 / 9	0 / 5	0 / 12	3 / 11	7 / 31	1 / 7	0 / 11	1 / 8	0 / 3
1M, oxidation	0 / 1	0 / 3	0 / 2	1 / 3	1 / 7	0 / 4	0 / 1	0 / 2	0 / 1	-
5M, oxidation	0 / 1	1 / 3	1 / 2	1 / 3	2 / 7	0 / 4	0 / 1	0 / 2	0 / 1	-
39Y, oxidation	0 / 11	0 / 16	0 / 11	0 / 16	0 / 31	1 / 13	0 / 9	0 / 13	0 / 9	0 / 5
50H, oxidation	2 / 26	0 / 28	2 / 26	2 / 89	5 / 107	0 / 31	0 / 14	0 / 15	0 / 11	0 / 7
116M, oxidation	0 / 23	6 / 23	2 / 25	10 / 38	11 / 58	0 / 31	0 / 11	0 / 16	0 / 11	0 / 4
127M, oxidation	0 / 6	1 / 9	0 / 5	3 / 12	1 / 11	4 / 31	0 / 7	0 / 11	0 / 8	0 / 3
136Y, oxidation	0 / 1	-	0 / 2	0 / 2	0 / 1	1 / 7	0 / 2	0 / 4	0 / 6	0 / 2

B

Amino acid residue and modification	JOS
33T, phosphorylation	2 / 12
39Y, phosphorylation	2 / 22
59T, phosphorylation	0 / 23
64T, phosphorylation	0 / 70
92T, phosphorylation	0 / 84
129S, phosphorylation	2 / 12
136Y, phosphorylation	3 / 12
6K, ubiqutination	6 / 10
12K, ubiqutination	-
21K, ubiquitination	4 / 9
23K, ubiquitination	-
45K, ubiquitination	1 / 12
60K, ubiquitination	2 / 23
80K, ubiquitination	0 / 70
102K, ubiquitination	0 / 43
N-terminal acetylation	10 / 10
12K, acetylation	-
23K, acetylation	-
32K, acetylation	0 / 10
34K, acetylation	1 / 12
45K, acetylation	6 / 12
58K, acetylation	0 / 58
60K, acetylation	6 / 23
80K, acetylation	0 / 70
97K, acetylation	0 / 15
102K, acetylation	0 / 43
12K, methylation	-
58K, methylation	3 / 59
60K, metylation	0 / 23
80K, methylation	0 / 73
96K, methylation	2 / 84
45K, dimethylation	0 / 12
58K, dimethylation	3 / 57
60K, dimethylation	0 / 26
80K, dimethylation	3 / 77
96K, dimethylation	1 / 86
34K, trimethylation	0 / 12
60K, trimethylation	2 / 26
80K, trimethylation	0 / 77
24Q, deamination	0 / 10
62Q, deamination	0 / 70
65N, deamination	3 / 70
72T, phosphorylation	0 / 70
79Q, deamination	0 / 70
99Q, deamidation	0 / 12
103N, deamidation	14 / 45
109Q, deamidation	4 / 43
122N, deamidation	9 / 19
134Q, deamidation	3 / 12
1M, oxidation	1 / 10
5M, oxidation	0 / 10
39Y, oxidation	2 / 22
50H, oxidation	5 / 58
116M, oxidation	3 / 40
127M, oxidation	0 / 12
136Y, oxidation	1 / 11

Phosphorylation
Ubiquitination
Acetylation
Methylation
Dimethylation
Trimethylation
Deamination
Oxidation

Fig. 1. Summary of PTMs in α-synucleopathy. (**A**) PTMs in MSA and DLB, and (**B**) PTMs in JOS. In these tables, the total number of peptides containing each amino acid residue and the number of peptides with modified residues are shown. Amino acid residues with detected modifications are colored. Y136 phosphorylation contained a peptide that was indistinguishable from Y133 phosphorylation.

including the processing pattern of the C-terminal fuzzy coat region, the pathological structures in JOS are different from those in ordinary DLB, and exhibit PTMs similar to those in MSA. This suggests that the nature of the PTMs, including the processing pattern of the C-terminal fuzzy coat region, depends on the filament core structure.

Although the filment core structures are partially similar and the PTMs pattern is very similar, GCIs are formed in MSA and Lewy bodies are formed in JOS. As regards the Lewy bodies and Lewy neurites formed in neurons, α-synuclein filaments with different filament core structures and different core-structure-related PTMs appear to form similar Lewy bodies and Lewy neurites in DLB and JOS. These results suggest that neither the core structure of α-synuclein filaments nor the PTMs are important for the formation of Lewy bodies and Lewy neurites. It remains to be determined what factors predominantly influence the formation of abnormal structures such as Lewy bodies and Lewy neurites.

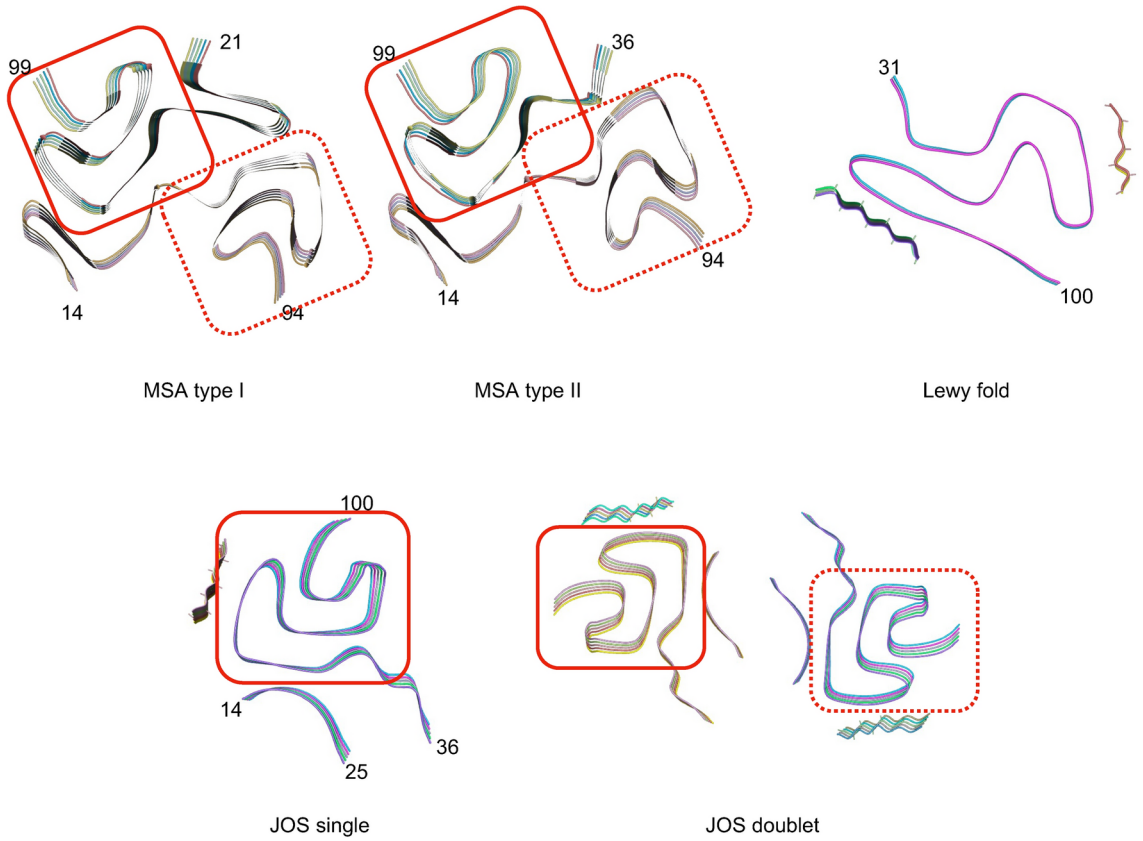

Fig. 2. Schematics of the filament core structures in JOS, Lewy, and MSA folds. The filaments from JOS are different from those of the Lewy fold observed in PD and DLB, and share only a partial similarity with the structure of the MSA fold (area surrounded by red lines).

Processing-derived fragment peptide	MSA-1	MSA-2	MSA-3	MSA-4	MSA-5	JOS	DLB-1	DLB-2	DLB-3	DLB-4	DLB-5
KDQLGKNEEGAPQ	O	O		O	O						
KDQLGKNEEGAPQEGILE						O					
KDQLGKNEEGAPQEGILED	O				O	O					
KDQLGKNEEGAPQEGILEDMPV						O					
KDQLGKNEEGAPQEGILEDMPVD	O	O	O	O	O	O	O	O	O	O	O
KDQLGKNEEGAPQEGILEDMPVDPD						O					
KDQLGKNEEGAPQEGILEDMPVDPDN	O	O	O	O	O	O	O		O		
DQLGKNEEGAPQEGILEDMPVD			O		O	O	O		O		
NEEGAPQEGILED						O					
NEEGAPQEGILEDMPVD				O		O	O	O	O		
NEEGAPQEGILEDMPVDPDN						O					

Table 1. Detected processing-derived fragment peptides. O: detected, Blank : not detected.

Methods

Extraction of α-synuclein filaments

Brain samples from five cases of MSA, 5 cases of DLB and one case of JOS (for details, see Table 2) were obtained from the Brain Banks in Tokyo Metropolitan Geriatric Hospital & Institute of Gerontology, National Center of Neurology and Psychiatry, Aichi Medical University, Sagamihara National Hospital and Mihara Memorial Hospital, and were analyzed as described elsewhere[42,51]. Briefly, the brain samples (0.5–1.0 g) were homogenized in 10 ml of homogenization buffer (10 mM Tris–HCl, pH 7.5, containing 0.8 M NaCl, 1 mM EGTA, 1 mM dithiothreitol). Sarkosyl was added to the homogenates (final concentration: 2%), which were then incubated for 30 min at 37 °C and centrifuged at 20,000 g for 10 min at 25 °C. The supernatants were centrifuged at 100,000 g for 20 min at 25 °C, then the pellets were washed with sterile saline and centrifuged at 100,000 g for 20 min. The resulting pellets were used as Sarkosyl-insoluble fraction, containing α-synuclein filaments [42]. This study was

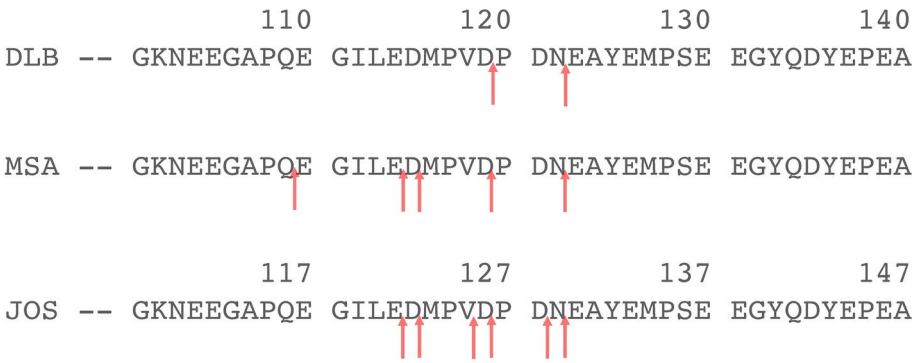

Fig. 3. Schematic drawing of C-terminal processing sites in JOS, DLB and MSA. The C-terminal processing sites of MSA and JOS are similar, and are different from that of DLB.

Case No	Age at death	Gender	Disease duration (year)	Neuropathological diagnosis
MSA1	85	F	9	MSA-P
MSA2	68	F	18	MSA-C
MSA3	59	M	9	MSA-C
MSA4	64	M	10	MSA-C
MSA5	70	M	19	MSA-C
JOS	13	F	2	DLB
DLB1	59	M	10	DLB
DLB2	79	M	4	DLB
DLB3	74	M	18	DLB
DLB4	84	M	5	DLB
DLB5	90	M	4	DLB
Control1	81	M	0	Normal
Control2	60	M	0	Nomal

Table 2. Description of the cases used PTMs analysis.

approved by the research ethics committee of Tokyo Metropolitan Institute of Medical Science (number: 21–1), and carried out in accordance with the approved guidelines. Informed consent for brain donation had been obtained from all subjects.

LC–MS/MS analysis of PTMs

Sarkosyl-insoluble fractions containing α-synuclein filaments were treated with 70% formic acid for 1 h at room temperature, then diluted in water and dried. For trypsin digestion, 50 mM triethylammonium bicarbonate and 1 μg of trypsin/Lys-C mix (Promega) were added, and the mixtures were incubated at 37 °C for 20 h. After tryptic digestion, 2 micro L of 100 mM DTT was added, and the mixture was incubated at 100 °C for 5 min, dried and stored at − 80 °C until assay[42,51].

Stored samples were thawed, resuspended in 0.1% formic acid and introduced into a nano-flow HPLC system, EASY-nLC 1200 (Thermo Fisher Scientific Inc., Waltham, USA). A packed nano-capillary column NTCC-360/75-3-123 (0.075 mm I.D. × 125 mm L, particle diameter 3 μm, Nikkyo Technos Co., Ltd., Tokyo, Japan) was used at a flow rate of 300 nl/min with a 2–80% linear gradient of acetonitrile for 80 min. Eluted peptides were directly detected with an ion trap mass spectrometer, QExactive HF (Thermo Fisher Scientific Inc., Waltham, USA). For ionization a spray voltage of 2.0 kV and capillary temperature of 250 °C were used. The mass acquisition method consisted of one full MS survey scan with an Orbitrap resolution of 60,000 followed by an MS/MS scan of the most abundant precursor ions from the survey scan with an Orbitrap resolution of 15,000. Dynamic exclusion for the MS/MS was set to 30 s. An MS scan range of 350–1800 m/z was employed in the positive ion mode, followed by data-dependent MS/MS using the HCD operating mode on the top 15 ions in order of abundance. The data were analyzed with Proteome Discoverer (Thermo Fisher Scientific Inc., Waltham, USA), Mascot software (Matrix Science Inc., Boston, USA) and Scaffold software (Proteome Software, Inc., Oregon, USA). Swissprot and GenBank databases were used[51].

Data availability

All raw data used for figure and table generation in this study can be obtained by contacting the corresponding author. Mass spectrometry data can be obtained from jPOSTrepo (Japan ProteOme STandard Repository), which is a certificated member of the ProteomeXchange Consortium under the ID number PXD018434.

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References

1. George, J. M., Jin, H., Woods, W. S. & Clayton, D. F. Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. *Neuron* **15**, 361–372. https://doi.org/10.1016/0896-6273(95)90040-3 (1995).
2. Perrin, R. J., Woods, W. S., Clayton, D. F. & George, J. M. Interaction of human alpha-Synuclein and Parkinson's disease variants with phospholipids. Structural analysis using site-directed mutagenesis. *J. Biol. Chem.* **275**, 34393–34398. https://doi.org/10.1074/jbc.M004851200 (2000).
3. George, J. M. The synucleins. *Genome Biol.* https://doi.org/10.1186/gb-2001-3-1-reviews3002 (2002).
4. Gilmozzi, V. *et al.* Interaction of Alpha-Synuclein with lipids: Mitochondrial cardiolipin as a critical player in the pathogenesis of Parkinson's disease. *Front. Neurosci.* **14**, 578993. https://doi.org/10.3389/fnins.2020.578993 (2020).
5. Ueda, K. et al. Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 11282-11286, https://doi.org/10.1073/pnas.90.23.11282 (1993).
6. Li, H. T., Du, H. N., Tang, L., Hu, J. & Hu, H. Y. Structural transformation and aggregation of human alpha-synuclein in trifluoroethanol: non-amyloid component sequence is essential and beta-sheet formation is prerequisite to aggregation. *Biopolymers* **64**, 221–226. https://doi.org/10.1002/bip.10179 (2002).
7. Giasson, B. I., Murray, I. V., Trojanowski, J. Q. & Lee, V. M. A hydrophobic stretch of 12 amino acid residues in the middle of alpha-synuclein is essential for filament assembly. *J. Biol. Chem.* **276**, 2380–2386. https://doi.org/10.1074/jbc.M008919200 (2001).
8. Li, W. W. *et al.* Localization of alpha-synuclein to mitochondria within midbrain of mice. *Neuroreport* **18**, 1543–1546. https://doi.org/10.1097/WNR.0b013e3282f03db4 (2007).
9. Post, M. R., Lieberman, O. J. & Mosharov, E. V. Can interactions between alpha-synuclein, dopamine and calcium explain selective neurodegeneration in Parkinson's Disease?. *Front. Neurosci.* **12**, 161. https://doi.org/10.3389/fnins.2018.00161 (2018).
10. Vilar, M. et al. The fold of alpha-synuclein fibrils. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 8637-8642, https://doi.org/10.1073/pnas.0712179105 (2008).
11. Lautenschlager, J. *et al.* C-terminal calcium binding of alpha-synuclein modulates synaptic vesicle interaction. *Nat. Commun.* **9**, 712. https://doi.org/10.1038/s41467-018-03111-4 (2018).
12. Goedert, M., Jakes, R. & Spillantini, M. G. The synucleinopathies: Twenty years on. *J. Parkinsons Dis.* **7**, S51–S69. https://doi.org/10.3233/JPD-179005 (2017).
13. Baba, M. *et al.* Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. *Am. J. Pathol.* **152**, 879–884 (1998).
14. Spillantini, M. G. *et al.* Alpha-synuclein in Lewy bodies. *Nature* **388**, 839–840. https://doi.org/10.1038/42166 (1997).
15. Wakabayashi, K., Yoshimoto, M., Tsuji, S. & Takahashi, H. Alpha-synuclein immunoreactivity in glial cytoplasmic inclusions in multiple system atrophy. *Neurosci. Lett.* **249**, 180–182. https://doi.org/10.1016/s0304-3940(98)00407-8 (1998).
16. Polymeropoulos, M. H. *et al.* Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* **276**, 2045–2047. https://doi.org/10.1126/science.276.5321.2045 (1997).
17. Singleton, A. B. *et al.* alpha-Synuclein locus triplication causes Parkinson's disease. *Science* **302**, 841. https://doi.org/10.1126/science.1090278 (2003).
18. Chartier-Harlin, M. C. *et al.* Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet* **364**, 1167–1169. https://doi.org/10.1016/S0140-6736(04)17103-1 (2004).
19. Ibanez, P. *et al.* Causal relation between alpha-synuclein gene duplication and familial Parkinson's disease. *Lancet* **364**, 1169–1171. https://doi.org/10.1016/S0140-6736(04)17104-3 (2004).
20. Singleton, A. & Gwinn-Hardy, K. Parkinson's disease and dementia with Lewy bodies: A difference in dose?. *Lancet* **364**, 1105–1107. https://doi.org/10.1016/S0140-6736(04)17117-1 (2004).
21. Zarranz, J. J. *et al.* The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Ann. Neurol.* **55**, 164–173. https://doi.org/10.1002/ana.10795 (2004).
22. Hoffman-Zacharska, D. *et al.* Novel A18T and pA29S substitutions in alpha-synuclein may be associated with sporadic Parkinson's disease. *Parkinsonism Relat. Disord.* **19**, 1057–1060. https://doi.org/10.1016/j.parkreldis.2013.07.011 (2013).
23. Kruger, R. *et al.* Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat. Genet.* **18**, 106–108. https://doi.org/10.1038/ng0298-106 (1998).
24. Proukakis, C., Houlden, H. & Schapira, A. H. Somatic alpha-synuclein mutations in Parkinson's disease: Hypothesis and preliminary data. *Mov. Disord.* **28**, 705–712. https://doi.org/10.1002/mds.25502 (2013).
25. Appel-Cresswell, S. *et al.* Alpha-synuclein p.H50Q, a novel pathogenic mutation for Parkinson's disease. *Mov. Disord.* **28**, 811–813. https://doi.org/10.1002/mds.25421 (2013).
26. Proukakis, C. *et al.* A novel alpha-synuclein missense mutation in Parkinson disease. *Neurology* **80**, 1062–1064. https://doi.org/10.1212/WNL.0b013e31828727ba (2013).
27. Lesage, S. *et al.* G51D alpha-synuclein mutation causes a novel parkinsonian-pyramidal syndrome. *Ann. Neurol.* **73**, 459–471. https://doi.org/10.1002/ana.23894 (2013).
28. Ghosh, D. *et al.* The newly discovered Parkinson's disease associated Finnish mutation (A53E) attenuates alpha-synuclein aggregation and membrane binding. *Biochemistry* **53**, 6419–6421. https://doi.org/10.1021/bi5010365 (2014).
29. Pasanen, P. *et al.* Novel alpha-synuclein mutation A53E associated with atypical multiple system atrophy and Parkinson's disease-type pathology. *Neurobiol Aging* **35**(2180), e2181-2185. https://doi.org/10.1016/j.neurobiolaging.2014.03.024 (2014).
30. Stefanis, L. alpha-synuclein in Parkinson's disease. *Cold Spring Harb. Perspect Med.* **2**, a009399. https://doi.org/10.1101/cshperspect.a009399 (2012).
31. Conway, K. A., Harper, J. D. & Lansbury, P. T. Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease. *Nat. Med.* **4**, 1318–1320. https://doi.org/10.1038/3311 (1998).
32. Choi, W. *et al.* Mutation E46K increases phospholipid binding and assembly into filaments of human alpha-synuclein. *FEBS Lett.* **576**, 363–368. https://doi.org/10.1016/j.febslet.2004.09.038 (2004).
33. Ono, K., Ikeda, T., Takasaki, J. & Yamada, M. Familial Parkinson disease mutations influence alpha-synuclein assembly. *Neurobiol. Dis.* **43**, 715–724. https://doi.org/10.1016/j.nbd.2011.05.025 (2011).
34. Ghosh, D. *et al.* The Parkinson's disease-associated H50Q mutation accelerates alpha-Synuclein aggregation in vitro. *Biochemistry* **52**, 6925–6927. https://doi.org/10.1021/bi400999d (2013).
35. Yonetani, M. *et al.* Conversion of wild-type alpha-synuclein into mutant-type fibrils and its propagation in the presence of A30P mutant. *J. Biol. Chem.* **284**, 7940–7950. https://doi.org/10.1074/jbc.M807482200 (2009).
36. Spillantini, M. G. *et al.* Filamentous alpha-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies. *Neurosci. Lett.* **251**, 205–208. https://doi.org/10.1016/s0304-3940(98)00504-7 (1998).
37. Spillantini, M. G., Crowther, R. A., Jakes, R., Hasegawa, M. & Goedert, M. alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 6469-6473, https://doi.org/10.1073/pnas.95.11.6469 (1998).
38. Fujiwara, H. *et al.* alpha-Synuclein is phosphorylated in synucleinopathy lesions. *Nat. Cell. Biol.* **4**, 160–164. https://doi.org/10.1038/ncb748 (2002).

39. Hasegawa, M. *et al.* Phosphorylated alpha-synuclein is ubiquitinated in alpha-synucleinopathy lesions. *J. Biol. Chem.* **277**, 49071–49076. https://doi.org/10.1074/jbc.M208046200 (2002).
40. Neumann, M. *et al.* Misfolded proteinase K-resistant hyperphosphorylated alpha-synuclein in aged transgenic mice with locomotor deterioration and in human alpha-synucleinopathies. *J. Clin. Invest.* **110**, 1429–1439. https://doi.org/10.1172/JCI15777 (2002).
41. Yang, Y. *et al.* Structures of alpha-synuclein filaments from human brains with Lewy pathology. *Nature* **610**, 791–795. https://doi.org/10.1038/s41586-022-05319-3 (2022).
42. Schweighauser, M. *et al.* Structures of alpha-synuclein filaments from multiple system atrophy. *Nature* **585**, 464–469. https://doi.org/10.1038/s41586-020-2317-6 (2020).
43. Zhang, S. *et al.* Post-translational modifications of soluble alpha-synuclein regulate the amplification of pathological alpha-synuclein. *Nat. Neurosci.* **26**, 213–225. https://doi.org/10.1038/s41593-022-01239-7 (2023).
44. Schmid, A. W., Fauvet, B., Moniatte, M. & Lashuel, H. A. Alpha-synuclein post-translational modifications as potential biomarkers for Parkinson disease and other synucleinopathies. *Mol. Cell Proteomics* **12**, 3543–3558. https://doi.org/10.1074/mcp.R113.032730 (2013).
45. Magalhaes, P. & Lashuel, H. A. Opportunities and challenges of alpha-synuclein as a potential biomarker for Parkinson's disease and other synucleinopathies. *NPJ. Parkinsons Dis.* **8**, 93. https://doi.org/10.1038/s41531-022-00357-0 (2022).
46. Yang, Y. *et al.* New SNCA mutation and structures of alpha-synuclein filaments from juvenile-onset synucleinopathy. *Acta Neuropathol.* **145**, 561–572. https://doi.org/10.1007/s00401-023-02550-8 (2023).
47. Takao, M. *et al.* Early-onset dementia with Lewy bodies. *Brain Pathol.* **14**, 137–147. https://doi.org/10.1111/j.1750-3639.2004.tb00046.x (2004).
48. Iwata, A. *et al.* Alpha-synuclein degradation by serine protease neurosin: Implication for pathogenesis of synucleinopathies. *Hum. Mol. Genet.* **12**, 2625–2635. https://doi.org/10.1093/hmg/ddg283 (2003).
49. Mishizen-Eberz, A. J. *et al.* Cleavage of alpha-synuclein by calpain: Potential role in degradation of fibrillized and nitrated species of alpha-synuclein. *Biochemistry* **44**, 7818–7829. https://doi.org/10.1021/bi047846q (2005).
50. Kasai, T. *et al.* Cleavage of normal and pathological forms of [alpha]-synuclein by neurosin in vitro. *Neurosci. Lett.* **436**, 52–56. https://doi.org/10.1016/j.neulet.2008.02.057 (2008).
51. Kametani, F. *et al.* Comparison of common and disease-specific post-translational modifications of pathological tau associated with a wide range of tauopathies. *Front. Neurosci.* **14**, 581936. https://doi.org/10.3389/fnins.2020.581936 (2020).

Author contributions

F.K. and M.H. desighed the research. M.T., T.M., K.H., M.Y., Y.S., and S.M. provided brain samples. F.K. and M.H. performed the biochemical analysis and F.K. and M.T. performed the mass spectrometry analysis. F.K. wrote the paper. All authors read and approved the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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