

# Atypical multiple system atrophy is a new subtype of frontotemporal lobar degeneration: frontotemporal lobar degeneration associated with $\alpha$ -synuclein

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**Abstract** Multiple system atrophy (MSA) is a sporadic neurodegenerative disease clinically characterized by cerebellar signs, parkinsonism, and autonomic dysfunction. Pathologically, MSA is an  $\alpha$ -synucleinopathy affecting striatonigral and olivopontocerebellar systems, while neocortical and limbic involvement is usually minimal. In this study, we describe four patients with atypical MSA with clinical features consistent with frontotemporal dementia (FTD), including two with corticobasal syndrome, one with progressive non-fluent aphasia, and one with behavioral variant FTD. None had autonomic dysfunction. All had frontotemporal atrophy and severe limbic  $\alpha$ -synuclein neuronal pathology. The neuronal inclusions were heterogeneous, but included Pick body-like inclusions. The latter were strongly associated with neuronal loss in the hippocampus and amygdala. Unlike typical Pick bodies, the neuronal

inclusions were positive on Gallyas silver stain and negative on tau immunohistochemistry. In comparison to 34 typical MSA cases, atypical MSA had significantly more neuronal inclusions in anteromedial temporal lobe and limbic structures. While uncommon, our findings suggest that MSA may present clinically and pathologically as a frontotemporal lobar degeneration (FTLD). We suggest that this may represent a novel subtype of FTLD associated with  $\alpha$ -synuclein (FTLD-synuclein).

**Keywords** Multiple system atrophy · Frontotemporal lobar degeneration · Neuropathology · Pick body-like inclusions ·  $\alpha$ -Synuclein

## Introduction

Multiple system atrophy (MSA) is a sporadic, adult-onset, neurodegenerative disease characterized clinically by cerebellar ataxia, parkinsonism and autonomic dysfunction with variable pyramidal signs [6, 21]. MSA encompasses three disorders that were at one time considered separate entities based on differing pathologic and clinical features: olivopontocerebellar atrophy (OPCA), striatonigral degeneration (SND), and Shy-Drager syndrome (SDS) [10]. In 1989, Papp and Lantos [30] described argyrophilic glial cytoplasmic inclusions (GCI) in oligodendrocytes in MSA, and GCI are now considered the pathognomonic lesions of MSA. The discovery that GCI were composed of  $\alpha$ -synuclein led to classification of MSA as an  $\alpha$ -synucleinopathy, a designation shared with Lewy body disease (LBD) presenting clinically as Parkinson's disease or dementia with Lewy bodies [8, 36, 38, 43].

Glial cytoplasmic inclusions can be found throughout the central nervous system, being most numerous in

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striatonigral and olivopontocerebellar systems, areas that also have variable neuronal loss. Ozawa and co-workers [29] showed that density of GCI was correlated with both disease duration and severity of neuronal loss, indicating that GCI are likely an important factor in neurodegeneration in MSA. In addition to GCI,  $\alpha$ -synuclein accumulates in cytoplasm, nuclei, and cell processes of neurons as neuronal cytoplasmic inclusions (NCI), neuronal intranuclear inclusions (NII), and dystrophic neurites [1, 23]. In typical MSA, NCI and dystrophic neurites are most often detected in the putamen, pontine nuclei, and inferior olivary nucleus [17, 27, 45], while limbic structures (e.g., hippocampus and amygdala) are affected to a lesser degree. The relationship between NCI and neurodegeneration is not clearly understood.

Neurodegeneration in neocortex and limbic structures is usually minimal in MSA. While motor cortex and supplementary motor cortex often have many GCI, neuronal loss is minimal [42]. There are a few reports of MSA with severe frontal or temporal lobe atrophy and numerous NCI [13, 16, 19, 31, 32, 46], but this is better recognized in Japan and is under recognized in Europe and North America. In the present study, we describe clinical and pathological features of four MSA patients who presented with FTD clinical syndromes associated with frontotemporal and limbic system degeneration. We also compare the frequency and severity of NCI in neocortical and limbic areas of these four cases to those found in 34 typical MSA cases.

## Materials and methods

### Case material

Four atypical MSA patients were included in this study. Two were from a series of brains submitted for review or consultation to the neuropathology laboratory at the Mayo Clinic in Jacksonville, Florida (MCJ), between 1997 and 2014 (Cases 1 and 2). Out of 124 MSA cases accessioned during that time period, only these two had frontotemporal atrophy with numerous limbic  $\alpha$ -synuclein NCI. The other two patients were from the University of Rochester in New York (Case 3) and the University of Texas Southwestern Medical Center (Case 4). Paraffin-embedded blocks from these two brains were available for histological examination and semi-quantitative evaluation. The four atypical MSA cases were compared to 34 typical MSA cases using semiquantitative methods.

### Neuropathologic assessment

Formalin-fixed and paraffin-embedded sections from cortical, subcortical, brainstem, and cerebellar regions were

stained with hematoxylin and eosin (H&E), a silver stain (Gallyas), and thioflavin-S for fluorescent microscopy. The density of senile plaques and neurofibrillary tangles (NFT) were assessed with thioflavin-S fluorescent microscopy and used to assign Thal amyloid phase [37] and Braak NFT stage [4], respectively. Luxol fast blue stain was used to assess white matter integrity on sections of anteromedial temporal lobe. Immunohistochemistry was performed on a DAKO Autostainer (Universal Staining System, Carpinteria, CA, USA) with the following antibodies:  $\alpha$ -synuclein (NACP, 1:3000, rabbit polyclonal, Mayo Clinic [2, 12]), phosphorylated tau (CP13, 1:1000, mouse monoclonal, gift from Dr. Peter Davies, Feinstein Institute for Medical Research, North Shore/Long Island), and TDP-43 (pS409/410, 1:5000 mouse monoclonal, Cosmo Bio USA, Carlsbad, CA or MC2085 [47], 1:2500 rabbit polyclonal, from Leonard Petrucelli, Mayo Clinic).

Sections of hippocampus were studied with double-labeling immunohistochemistry by combining  $\alpha$ -synuclein polyclonal antibody (NACP) and phosphorylated tau monoclonal antibody (CP13 or PHF-1, 1:1000, mouse monoclonal, gift from Dr. Peter Davies, Feinstein Institute for Medical Research, North Shore/Long Island) or A $\beta$  monoclonal antibody (4G8, 1:50,000, mouse monoclonal, BioSource International/Invitrogen Corp., Carlsbad, CA).

### Semi-quantitative assessment of $\alpha$ -synuclein immunohistochemistry

A semi-quantitative assessment of  $\alpha$ -synuclein pathology was performed in the following regions: cerebral cortex—middle frontal gyrus, superior temporal gyrus, parahippocampal gyrus, and cingulate gyrus; subcortical areas—hippocampus, amygdala, hypothalamus, and putamen; brainstem—substantia nigra, pontine nuclei, inferior olivary nucleus, medullary tegmentum, and pyramidal tract; and cerebellar white matter. The densities of NCI and GCI were scored separately using a 4-point scale: 0 = none, 1 = sparse to mild, 2 = moderate, 3 = severe. In addition, the density of NCI in select brain regions (hippocampus, hypothalamus, and inferior olivary nucleus) was assessed in 34 typical MSA cases for comparison to atypical MSA.

### Immunoelectron microscopy

Formalin-fixed tissues from subiculum and amygdala from case 1 and from hippocampus and cerebellum from case 2 were processed for post-embedding immunogold electron microscopy as previously reported [23, 41]. Rabbit polyclonal antibody to  $\alpha$ -synuclein and a mouse monoclonal antibody to phosphorylated tau (CP13 or PHF-1) were used

for immuno-EM. Stained ultrathin sections were examined on a Philips 208S electron microscope (Phillips, Amsterdam, NL) fitted with a Gatan 831 Orius digital camera (Gatan, Inc., Pleasanton, CA, USA).

### Genetic analysis

Genomic DNA was extracted from frozen brain tissue. For direct sequence analysis, each of the coding exons of *SNCA* (exons 2 through 6) was amplified by polymerase chain reaction (PCR) using exon-specific primers for *SNCA* (available upon request). Sequencing reactions were performed using BigDye Terminator chemistry. Genomic sequences were generated on an ABI 3730XL DNA Analyzer and read with SeqScape Software v2.5 (Life Technologies, Carlsbad, CA, USA). For *SNCA* genomic dosage analysis, TaqMan Copy Number assay targeted to exon 6 was used (details available on request). TaqMan Copy Number Reference Assay RNase P (human) was used as an endogenous control. Quantitative PCR was carried out using TaqMan expression chemistry protocol. All assays were performed in triplicate on the ABI 7900HT Fast

Real-Time PCR System and analyzed with ABI SDS 2.2.2 Software (Life Technologies).

### Statistical analysis

SigmaPlot 12.0 (Systat Software, Inc., San Jose, CA, USA) statistical software was used to analyze data. Due to the small sample size, non-parametric methods (Mann–Whitney rank-sum test) were used for group comparisons of continuous variables. Fisher's exact test was used for group comparison of categorical data. Statistical significance was considered  $P < 0.05$ .

## Results

### Clinical features

Clinical features of atypical MSA cases are summarized in Table 1. The antemortem clinical diagnosis for Cases 1 and 3 was corticobasal syndrome (CBS) [3, 20], while case 2 was progressive non-fluent aphasia (PNFA) [11], and Case

**Table 1** Clinical features of atypical MSA cases

	Case 1	Case 2	Case 3	Case 4
Sex	Female	Female	Female	Female
Age at death, years	91	88	70	73
Age at onset, years	88	70	67	66
Duration of illness, years	3	18	3	7
Family history	None	None	None	n.a.
Initial symptoms	Dystonia	Aphasia	Abnormal tongue movement	Memory impairment, depression, personality change
Clinical diagnosis	CBS	PNFA	CBS	bvFTD
Dysarthria	+	+	+	
Dysphagia	+		+	
Urinary problems	+	+		
Parkinsonism	+		+	+
Pyramids sign	+		+	
Dystonia	+			
Myoclonus			+	
Apraxia			+	
Involuntary movements			+	
Memory impairment		+	+	+
Depression				+
Personality change		+		+
Overeating				+
Aphasia		+		
Cerebellar signs			+	

A particular clinical symptom or sign is displayed as (+) if specifically stated in the clinical records. Otherwise, they are displayed as blank space since it is difficult to conclude that a symptom or sign was absent from retrospective medical records

CBS corticobasal syndrome, PNFA progressive non-fluent aphasia, bvFTD behavior variant frontotemporal dementia, n.a. not available

4 was behavioral variant frontotemporal dementia (bvFTD) [26].

#### Case 1

This 91-year-old right-handed woman had a 3-year history of progressive motor dysfunction. She was living independently and was asymptomatic until the age of 87 years. At age 88 years, she noticed her left hand curling up, which was thought to be dystonic posturing. The symptoms led to decreased ability to use her left arm. This progressed to her left leg. Eventually, she had left-side contractures. In the last year of her life, she developed similar problems on her right side. She had stiffness, difficulty walking, and tremors. She had dysphagia and recurrent bladder infections. On examination, she had marked weakness in all her extremities, and she had increased tone and rigidity, as well as dystonia. She had mild dysarthria and unintelligible speech. Her reflexes were brisk in both upper extremities, but they could not be elicited in either of the lower extremities. Her toes were mute on testing for Babinski sign, and there was no clonus. There was no upward or downward gaze palsy. She had no documented autonomic dysfunction. Her neurological diagnosis was CBS.

#### Case 2

This 88-year-old, right-handed woman had an 18-year history of progressive aphasia. When she initially presented at the age of 70 years, she had difficulty distinguishing the pronouns “he” and “she”. For the first 5 years, her illness remained mild. At age 75 years, her husband died, and she worsened. By age 79, she lost use of most nouns and adjectives. She was unable to write, although she could occasionally copy. Her verbal output was mildly decreased, but what she produced was generally garbled and incomprehensible. Her understanding of written language was better preserved. She was disinhibited and had some behavioral changes. She began to point out people inappropriately in public. She also started to collect sugar packets. At the age of 80 years, she developed a number of urinary tract infections. On examination at age 81 years, she was unable to give her name in response to a simple command, although she could remove her gloves and shoes without difficulty. Cognitive testing was compromised by her language deficits. On cranial nerve testing, there were no abnormalities, except mild dysarthria and unintelligible speech. No parkinsonism or cerebellar signs were noted, and she had no documented autonomic dysfunction. An MRI of the brain showed unilateral cortical atrophy, left-greater-than-right, anterior-greater-than-posterior, and temporal-greater-than-frontal atrophy. After this exam, her symptoms further progressed to terminal dementia. Her neurological diagnosis was PNFA.

#### Case 3

This 70-year-old woman had a 3-year history of progressive motor and cognitive dysfunction. At age 67, her daughter noticed that she would move her tongue abnormally. Six months later, she began to notice symptoms that affected her eating and speech. She began to stutter, especially when she was nervous. She developed twitching on the left side of her face. She had difficulty controlling her tongue and closing her eyes when she opened her mouth. She also had tremors in her left upper extremity and decreased fine motor movements in both upper extremities, with the left more affected than the right. Examination at the age of 68 years revealed orobuccal lingual apraxia, myoclonic jerks, involuntary movements, and cogwheel rigidity of both upper extremities, with the left more affected than the right. She also demonstrated frontal release signs, dysphasia, and intentional tremor. Her gait was slow, and she had decreased arm swing on the left side. In the final stage of her illness, her gait became unsteady. Her right extremities developed myoclonus. She had severe oral apraxia and bilateral facial myoclonus. She also had increased tone, throughout, with adventitious movements in all extremities, but mostly on the right side of her body. She had gait apraxia and severe instability while walking. She suffered from terminal dementia. She had no documented autonomic dysfunction. Her neurological diagnosis was CBS.

#### Case 4

This 73-year-old woman presented with a slowly- progressive memory decline that started when she was 66 years old. It was accompanied by depression and changes in her personality. Another notable feature was a change in her appetite; she developed significant overeating and weight gain. Toward the end of her life, she had abnormal gait and posture, as well as bradykinesia and cogwheel rigidity. Her symptoms progressed slowly over the years. She progressed to a point where she required assistance with activities of daily living, necessitating placement in an assisted-living facility. Ultimately, she needed skilled nursing. She had no documented autonomic dysfunction. Her neurological diagnosis was bvFTD.

### Pathological findings

#### Neuronal loss and gliosis

The distribution of cerebral atrophy and neuronal loss are shown in Table 2. In Case 1, cerebral atrophy was most marked in the frontal pole and the superior frontal gyrus, with less atrophy in the precentral gyrus. There was also peri-Sylvian atrophy affecting the inferior frontal lobe and

**Table 2** Pathological features of atypical MSA cases

	Case 1	Case 2	Case 3	Case 4
Brain weight, grams	1220	560	1170	1090
Cerebral atrophy	F > T	F < T	F, T, P	F, T
Braak NFT stage	III–IV	VI	II	I
Thal A $\beta$ phase	3	5	0	0
TDP-43 pathology	None	None	None	None
Neuronal loss and gliosis				
Hippocampal dentate fascia	–	+++	+	+++
Hippocampal CA1/subiculum	–	+++	++	+++
Amygdala	+	+++	n.a.	+++
Putamen	++	+++	+++	+
Substantia nigra	+	+++	++	+++
Pontine nuclei	–	+++	–	–
Inferior olivary nucleus	+	+++	–	–
Cerebellar granule cells	–	+	–	–
Purkinje cells	–	++	–	–
$\alpha$ -Synuclein-positive NCI				
Middle frontal cortex	+	+++	+++	+++
Superior temporal cortex	+	++	n.a.	n.a.
Cingulate cortex	+	+++	n.a.	n.a.
Hippocampal dentate fascia	+	+++	+++	+++
Hippocampal CA1/subiculum	+++	+++	+++	+++
Parahippocampal gyrus	+++	+++	+++	+++
Amygdala	+++	+++	n.a.	+++
Hypothalamus	+++	+++	+++	+++
Putamen	+++	+++	+++	+++
Substantia nigra	+	+	+++	++
Pontine nuclei	+++	+++	++	+++
Inferior olivary nucleus	+++	+++	–	+
$\alpha$ -Synuclein-positive GCI				
Frontal white matter	+	+	++	++
Hippocampus (alveus)	+	++	+++	+++
Amygdala	+	+	n.a.	++
Hypothalamus	+	+	+	+
Putamen	+++	+++	+++	+++
Pontine base	+++	+++	+	+++
Medullary tegmentum	+++	+++	+	+++
Pyramids	+++	++	+	++
Cerebellar white matter	+++	+++	+	+++

F frontal lobe, T temporal lobe, P parietal lobe, n.a. not available

the superior temporal lobe. In Cases 2 and 4, cerebral atrophy was most marked in the anterior frontal and temporal lobes (Fig. 1a, b). In Case 3, bilateral frontoparietal atrophy and softening of the right temporal lobe were remarkable. Marked medial temporal lobe atrophy, including the hippocampus and amygdala, was noted in three cases (Cases 2, 3, and 4) (Fig. 1c), but not in Case 1. Severe myelin pallor in the temporal lobe was noted in Case 2, but it

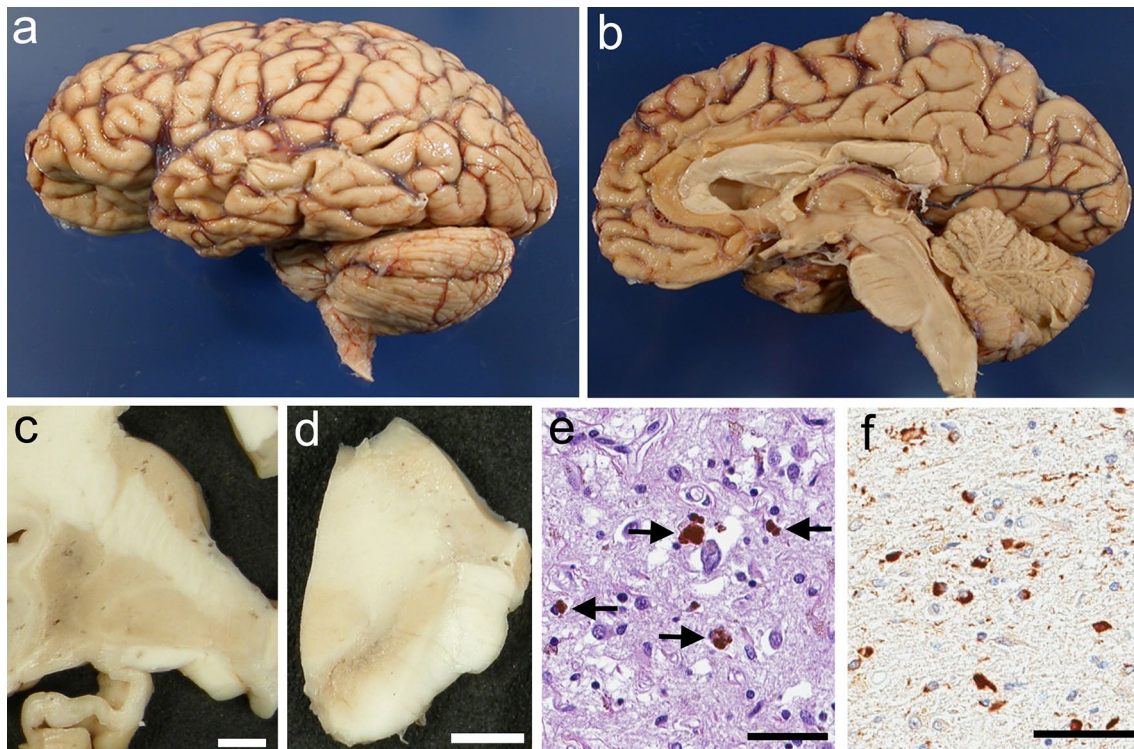
was mild in Cases 3 and 4. No myelin pallor was noted in Case 1. Marked atrophy and dark gray-brown discoloration of the putamen was noted in three cases (Cases 1, 2, and 3) (Fig. 1c), but it was mild in Case 4. Neuronal loss and gliosis were most marked in the lateral and posterior putamen, and it was accompanied by iron pigment (Fig. 1e). Marked decreased neuromelanin pigmentation was noted in the substantia nigra of all cases (Fig. 1d). Neuronal loss and gliosis with extraneuronal neuromelanin was most marked in ventrolateral cell groups. Marked atrophy of the pontine base and cerebellum was noted in Case 2, but not in the other three cases (Fig. 1b). Mild neuronal loss and Bergmann gliosis were noted in internal granule cell and Purkinje cell layers in Case 2, but not in the other three cases.

#### $\alpha$ -Synuclein neuronal pathology

The distribution and severity of  $\alpha$ -synuclein-positive NCI are shown in Table 2. In many areas, NCI were accompanied by variable numbers of dystrophic neurites. In the frontotemporal cortex, all cases had NCI and dystrophic neurites (Supplemental Fig. 1). The NCI were more frequent in the superficial and deep layers of the cortex. Some were visible on H&E stains as eosinophilic round inclusions, similar to Pick bodies.

In the limbic region, all cases had numerous NCI that were morphologically heterogeneous and included Pick body-like inclusions, as well as NFT-like and ring-shaped inclusions. The Pick body-like inclusions were most strongly associated with neuronal loss and gliosis. In Case 4 numerous Pick body-like inclusions were noted in the hippocampal dentate fascia (Fig. 2a). Similar to genuine Pick bodies some of these inclusions were visible on H&E stains (Fig. 2i). Numerous ring-shaped inclusions were noted in the dentate fascia in Case 3 (Fig. 2b). In Cases 3 and 4, numerous Pick body-like inclusions were noted in the hippocampal CA1 sector and subiculum (Fig. 2c, d). NFT-like inclusions were the predominant type of NCI in Case 1 (Fig. 2e). In addition, Case 1 had  $\alpha$ -synuclein immunopositive dystrophic neurites in the corona of senile plaques in the subiculum (Fig. 2f) and the amygdala. Case 2 had heterogeneous NCI, including Pick body-like inclusions, as well as many dystrophic neurites in the dentate fascia and pyramidal cell layer of the hippocampus. All cases had abundant NCI in the entorhinal cortex (Fig. 3g), amygdala (Fig. 3h), and hypothalamus. NCI were frequent in the posterior hypothalamus of three cases (Cases 2, 3, and 4) and mild in one case (Case 1). Severe neuronal pathology was noted in the anterior hypothalamus in Cases 1 and 2, but this region was not available for study in Cases 3 and 4.

In the putamen, all cases had abundant NCI and variable dystrophic neurites. Case 3 had abundant NCI in the



**Fig. 1** Macroscopic and microscopic findings in atypical MSA. Cerebral atrophy is marked in anterior frontal and temporal lobes (**a**). No atrophy is evident in the pons and cerebellum (**b**). Severe atrophy in the medial temporal lobe, including the hippocampus (**c**). Severe atrophy and *dark gray-brown* discoloration of the putamen (**c**). Marked decreased pigmentation of the substantia nigra (**d**). Severe neu-

ronal loss and gliosis in the putamen, accompanied by iron pigment (*arrows*) (**e**). Many  $\alpha$ -synuclein-positive glial cytoplasmic inclusions in the cerebellar white matter. Case 4 (**a**, **b**). Case 2 (**c–f**). H&E stain (**e**).  $\alpha$ -synuclein immunohistochemistry (**f**). Scale bars **c**; 5 cm, **d**; 500 mm, **e**, **f**; 50  $\mu$ m

substantia nigra, while they were less frequent in the other three cases. In the inferior olivary nucleus, abundant NCI were noted in Cases 1 and 2, but they were sparse in Cases 3 and 4.

#### *$\alpha$ -Synuclein glial pathology*

The distribution and severity of GCI are shown in Table 2. All cases had many GCI that were morphologically identical those in MSA. The regions with the highest density of GCI were the striatonigral and olivopontocerebellar systems. All cases had many GCI in the putamen. Many GCI were present in pontine base, medullary tegmentum, and cerebellar white matter (Fig. 1f), in Cases 1, 2 and 4, while they were sparse in Case 3. GCI were present in the neocortical and limbic structures, but they were generally sparse.

#### *Tau immunoreactivity for neuronal inclusions*

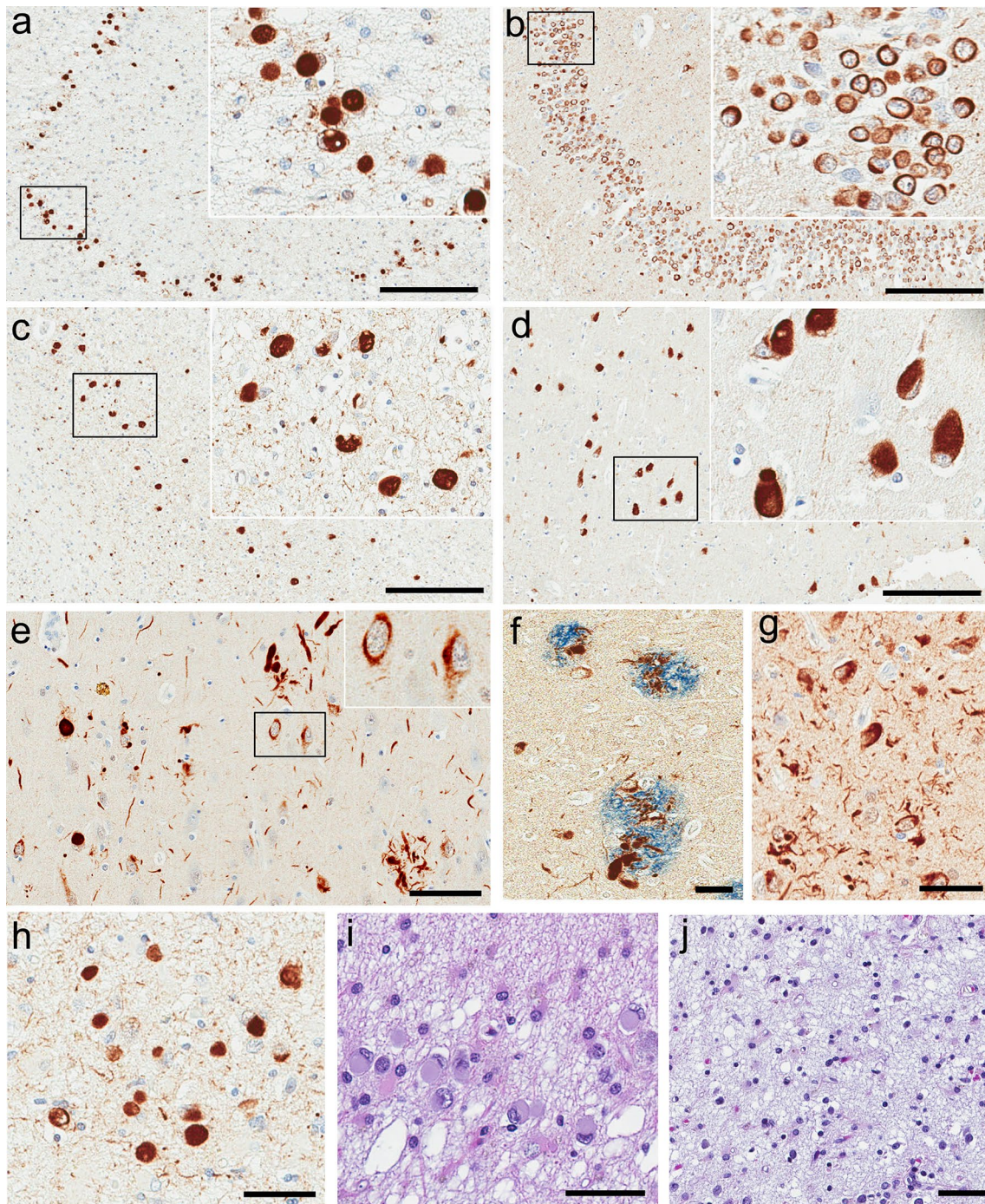
The  $\alpha$ -synuclein-positive NCI were intensely positive on Gallyas silver stains, while tau immunoreactivity was minimal. NCI that had tau immunoreactivity were variable with respect to number that were positive and the regions in

which they were found (Fig. 3). In Case 3, numerous ring-shaped (Fig. 3a–c) and Pick body-like inclusions (Fig. 3d–f) in the hippocampus were mostly negative for tau. In Case 4, some of the Pick body-like inclusions in the hippocampus were positive for tau, although the number was much less than those positive for  $\alpha$ -synuclein and Gallyas stains (Fig. 3g–i). Double immunohistochemistry using sections of the hippocampus revealed co-localization of  $\alpha$ -synuclein and tau in a subset of Pick body-like inclusions or NFT-like inclusions in three cases (Cases 1, 2 and 4) (Supplemental Fig. 2).

In the frontal cortex, almost no NCI were detected with tau immunohistochemistry in three cases (Cases 1, 3, and 4) (Fig. 4j–l). Case 2 had abundant neurofibrillary pathology in the frontal cortex that was a feature of concomitant AD-type pathology. In the putamen, pontine nuclei, and inferior olivary nucleus, almost no NCI were positive for tau in any of the cases.

#### *Immunoelectron microscopy*

Pick body-like inclusions were composed of granule-coated filaments randomly oriented. The filaments were

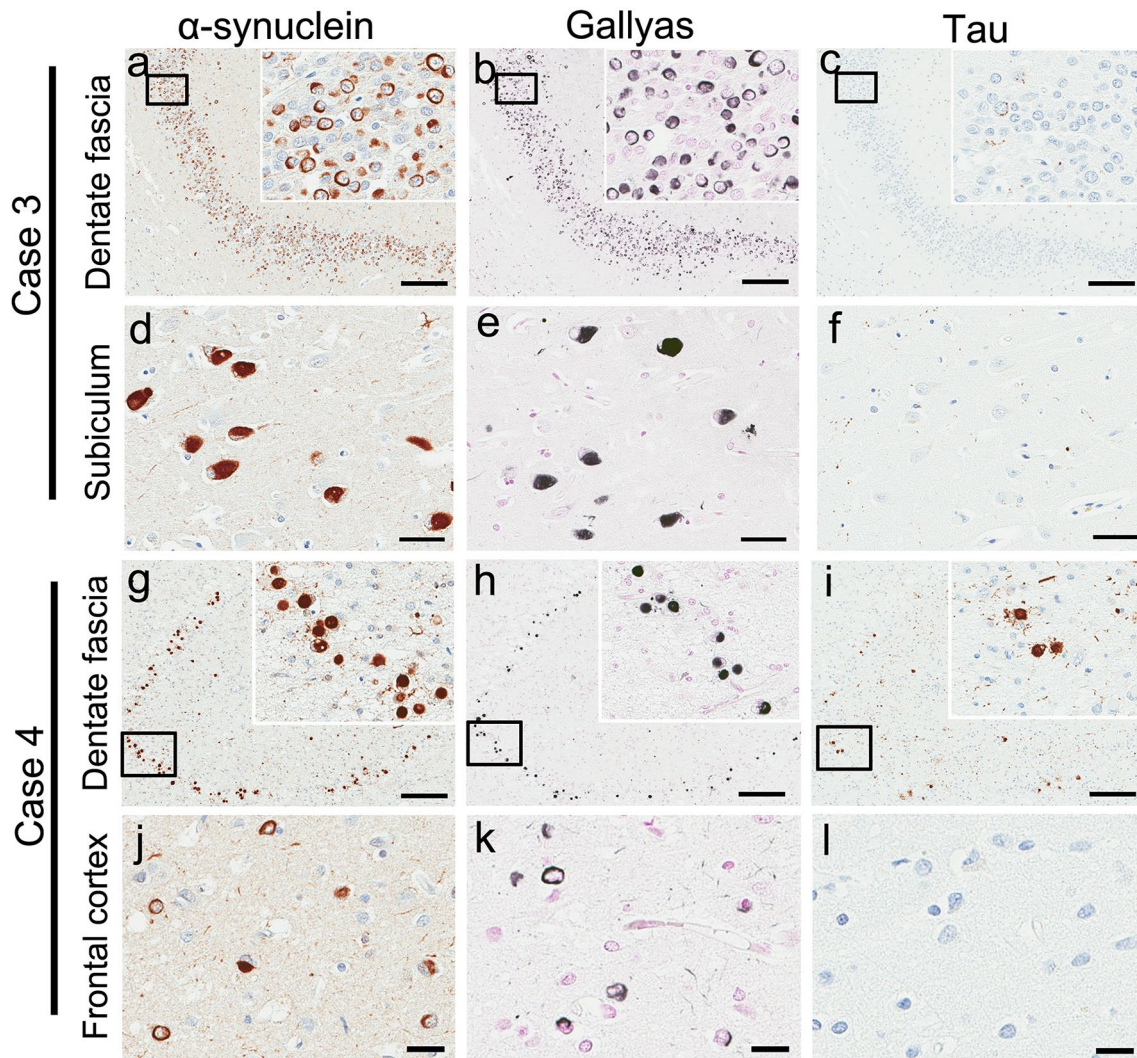


**Fig. 2** Limbic  $\alpha$ -synuclein neuronal pathology in atypical MSA. Many Pick body-like (a) and ring-shaped (b) inclusions in the hippocampal dentate fascia. Many Pick body-like inclusions in the subiculum (c, d). Many NFT-like inclusions and neurites in the subiculum (e). Inset in (e) shows NFT-like inclusions. Thick short neurites in senile plaques in the subiculum (f). Many NFT-like inclusions and neurites in the entorhinal cortex (g). Many Pick body-like inclusions

in the amygdala (h). Pick body-like inclusions in the dentate fascia are also visible on H&E stains (i). Severe neuronal loss and gliosis in the entorhinal cortex (j). Case 4 (a, c, h–j). Case 3 (b, d). Case 1 (e–g).  $\alpha$ -Synuclein immunohistochemistry (a–e, g, h). Double immunohistochemistry with  $\alpha$ -synuclein (brown) and amyloid- $\beta$  (blue) (f). H&E stain (i, j). Scale bars a–d; 400  $\mu$ m, e–j; 50  $\mu$ m

immunolabeled with  $\alpha$ -synuclein antibody. The nucleus was often pushed to the side and indented (Fig. 4a–c). Dystrophic neurites, including dystrophic neurites in

senile plaques (Supplemental Fig. 3), contained granule-coated  $\alpha$ -synuclein-positive filaments. When  $\alpha$ -synuclein and tau were found in the same neuron, as in Case 1,



**Fig. 3** Neuronal inclusions are intensely positive on  $\alpha$ -synuclein (a, d, g, j) and Gallyas silver stains (b, e, h, k), but tau immunoreactivity is variable among each case and region. In Case 3, almost no neuronal inclusions are positive on tau stains in the hippocampal dentate

fascia (c) or subiculum (f). In Case 4, some of neuronal inclusions are positive on tau stains in the hippocampal dentate fascia (i), while no neuronal inclusions are positive on tau stains in the frontal cortex (l). Scale bars a–c, g–i; 200  $\mu$ m, d–f, j–l; 50  $\mu$ m

$\alpha$ -synuclein filaments tended to be perinuclear, while tau formed discrete cytoplasmic filamentous aggregates (Supplemental Fig. 4). Tau-positive filaments were almost all straight filaments; only a few paired helical filaments were detected.

#### Additional pathologies

The severity of AD-type pathology is shown in Table 2. Case 2 had severe AD-type pathology (Braak NFT stage VI, Thal A $\beta$  phase 5), and Case 1 had mild AD-type pathology (Braak NFT stage III–IV, Thal A $\beta$  phase 3). Minimal NFT pathology and no SP were observed in Case 3 and Case 4. No TDP-43 pathology was observed in any of the cases.

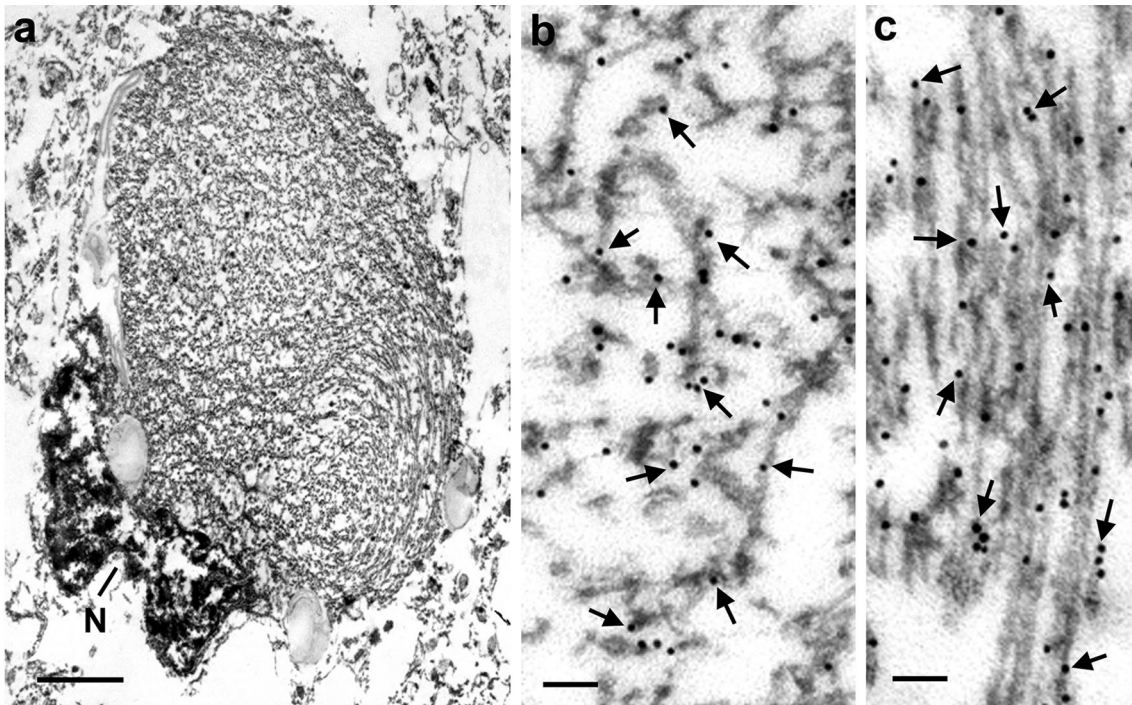
#### Genetic findings

For the two cases in which frozen tissue was available (Cases 1 and 2), whole gene sequencing of *SNCA* for point mutations and copy number variation was performed. Both cases were negative for *SNCA* point mutations and genomic multiplications.

#### Comparison of atypical MSA to typical MSA

The four atypical MSA cases were compared to 34 typical MSA cases with respect to frequency and density of NCI in limbic areas affected in atypical MSA (hippocampal dentate fascia, CA1/subiculum, and hypothalamus) and a region vulnerable to NCI in typical MSA (inferior





**Fig. 4** Electron micrograph of a Pick body-like inclusion that displaces the nucleus (N) to the periphery. The nucleus is highly indented and has condensed chromatin (a). Enlargement of central region shows randomly orientated granule-coated filaments heavily

labeled with  $\alpha$ -synuclein antibody. *Arrows* point to some of the 18 nm gold particles (b). Enlargement of peripheral region shows parallel granule-coated filaments. *Arrows* point to some gold particles (c). Case 2, hippocampus. *Scale bars* a; 1  $\mu$ m, b, c; 100 nm

olivary nucleus) (Table 3). All the atypical MSA cases were women, while women made up 44 % of the typical MSA group. The atypical MSA group had a significantly older median age at onset than the typical MSA group. There was no significant difference in the median brain weight, disease duration, Braak NFT stage, or Thal amyloid phase. NCI were often observed in the limbic regions in typical MSA, but they were very sparse compared to atypical MSA. In contrast, the frequency and severity of NCI in the inferior olivary nucleus were not different between atypical and typical MSA.

## Discussion

In this study, we describe four patients with atypical MSA who presented clinically as FTD, but without documented autonomic dysfunction, a requirement for diagnosis of clinically probable MSA [9]. All cases met pathological criteria for MSA, with striatonigral degeneration and variable olivopontocerebellar degeneration; all had pathognomonic  $\alpha$ -synuclein-positive GCI [21]. On the other hand, macroscopic and microscopic findings were suggestive of FTLD, except that neuronal inclusions were not composed of TDP-43 or tau, but rather  $\alpha$ -synuclein. The clinical syndromes included CBS, PNFA, and bvFTD, which

are clinical phenotypes of FTLD [15, 33, 35]. The association of Pick body-like NCI with neuronal loss suggests that cortical and limbic  $\alpha$ -synuclein neuronal pathology contributed to the atypical clinical presentations. There are limited reports of MSA presenting with FTD clinical syndromes [33].

Neuronal inclusions detected with  $\alpha$ -synuclein immunohistochemistry were most numerous in limbic and cortical regions and included Pick body-like, NFT-like, and ring-shaped inclusions. Of these various morphologic subtypes, Pick -body-like inclusions were most strongly associated with neuronal loss and gliosis, particularly in the hippocampus and amygdala. The neuronal inclusions in atypical MSA were easily distinguished from Lewy bodies in that they were intensely positive on Gallyas stains, while Lewy bodies are negative on Gallyas stains [39]. In addition, tau immunohistochemistry was helpful in distinguishing the Pick body-like and NFT-like inclusions from genuine Pick bodies [48] and NFT because most of the NCI in atypical MSA were negative. Furthermore, Pick bodies are usually negative on Gallyas stains [40]. These findings support the notion that atypical MSA is not MSA with co-existent Pick's disease, AD or LBD, but rather a variant of MSA [31, 32].

It is worth noting a previous case report of a 51-year-old woman with progressive dementia, psychosis and muscular

**Table 3** Comparison of atypical MSA to typical MSA

	Atypical MSA ( <i>n</i> = 4)	Typical MSA ( <i>n</i> = 34)	<i>P</i> value
Female	4/4 (100 %)	15/34 (44 %)	0.105
Brain weight, grams	1130 (693, 1208)	1180 (1100, 1325)	0.295
Age at death, years	81 (71, 90)	66 (62, 71)	0.015
Age at onset, years	69 (66, 84)	58 (53, 62)	0.010
Duration of illness, years	5 (3, 15)	8 (6, 10)	0.319
Braak NFT stage	III (I, V)	I (I, II)	0.085
Thal A $\beta$ phase	1.5 (0, 4.5)	2 (0, 2)	0.590
$\alpha$ -Synuclein-positive NCI			
Hippocampal dentate fascia			
Frequency	4/4 (100 %)	18/33 (55 %)	0.131
Scores	3 (1.5, 3)	1 (0, 1)	0.005
Hippocampal CA1/subiculum			
Frequency	4/4 (100 %)	22/33 (67 %)	0.296
Scores	3 (3, 3)	1 (0, 1)	<0.001
Hypothalamus			
Frequency	4/4 (100 %)	21/32 (66 %)	0.290
Scores	3 (3, 3)	1 (0, 2)	0.003
Inferior olivary nucleus			
Frequency	3/4 (75 %)	32/32 (100 %)	0.111
Scores	2 (0.25, 3)	2 (1, 3)	0.873

All data are displayed as median (25th, 75th, range), unless otherwise noted. Neuronal cytoplasmic inclusion (NCI) scores are assessed semi-quantitatively using a 4-point grading scale (0 = none, 1 = sparse to mild, 2 = moderate, 3 = severe)

rigidity with neuropathological features of both MSA and LBD [34]. This patient had frontotemporal and hippocampal atrophy with many  $\alpha$ -synuclein-positive, ring-shaped NCI in the hippocampal dentate fascia, similar to those in one of our patients (Case 3). These findings lead us to conclude that this case might be another example of atypical MSA. Unfortunately, clinical details were sparse making it difficult to know if she had features of FTD.

Co-localization of  $\alpha$ -synuclein and tau in a subset of Pick body-like and NFT-like inclusions was observed, which was consistent with a previous report of atypical MSA [31]. Co-localization of tau and  $\alpha$ -synuclein has been reported in sporadic LBD [14] and familial LBD due to mutations in *SNCA* [5, 7, 18, 25, 28]. Although the mechanism of abnormal tau accumulation in  $\alpha$ -synuclein inclusions is unknown, it may be noteworthy that  $\alpha$ -synuclein oligomers can seed tau aggregation *in vitro* [22].

In addition to the possible case noted above [34], six other atypical MSA cases have been reported—one from the United States (US) [13] and five from Japan [16, 19, 31, 32, 46] (Table 4). Pathologically, all cases had similar features, including frontotemporal atrophy and numerous

limbic NCI, including Pick body-like inclusions. Clinically, the course of the disease was different between US and Japanese cases. The median age of symptomatic onset was older in US than Japanese patients (70 vs. 53 years,  $P = 0.008$ ). The disease duration tended to be shorter in US than Japanese patients (3 vs. 15 years,  $P = 0.056$ ). Unlike the US cases, at least three Japanese cases had antemortem clinical diagnosis of MSA, although marked cerebral atrophy noted in the early stages of the disease process indicated that they were atypical MSA. It is possible that greater severity of cerebellar and brainstem pathology in the Japanese cases may have led to recognition of antemortem MSA. In the US cases, only one had cerebellar atrophy; while two cases had cerebellar signs, they occurred later in the disease. Memory impairment, which is not a characteristic feature of MSA [9], was frequent in US (3/5) and most Japanese cases (4/5), including cases with an antemortem diagnosis of MSA.

Autonomic dysfunction, such as orthostatic hypotension and syncope, were not described in the clinical records of any of our four cases, although they all had severe  $\alpha$ -synuclein neuronal pathology in the hypothalamus. The lack of autonomic dysfunction is at variance with a clinical diagnosis of MSA [9].

In comparison to typical MSA, limbic NCI were significantly more frequent and severe in atypical MSA. Only sparse NCI were detected in limbic structures in typical MSA [44]. Wang et al. reported that 55 % (17/31) of MSA cases had NCI in the hippocampus, including the dentate fascia. The density of limbic NCI in most of our typical MSA cases was very low; however, two cases had more abundant lesions. These two cases were not considered to be atypical MSA since they did not have cerebral atrophy or an FTD clinical syndrome. It will be important to examine the clinical impact of limbic  $\alpha$ -synuclein neuronal pathology in larger autopsy cohorts of pathologically confirmed MSA. The variable severity of limbic NCI in typical MSA cases suggests that atypical MSA is an extreme phenotype. We consider that numerous Pick body-like inclusions in hippocampus and amygdala associated with neuronal loss and atrophy are the most specific features of atypical MSA, although exceptional cases (Case 1) may have NFT-like inclusions, rather than Pick body-like inclusions.

The nosology of the disorder shared by the four patients we report is worth discussion. While pathologically they had the hallmark GCI of MSA, clinically they presented with FTD syndromes and they had frontotemporal degeneration with severe limbic and cortical  $\alpha$ -synuclein neuronal pathology. FTLN is currently classified based on the protein that accumulates within neuronal and glial lesions (e.g., FTLN-tau, FTLN-TDP, and FTLN-FUS). Atypical MSA does not fit into any of the current categories [24]. We propose a new category of FTLN for

**Table 4** Summary of previously reported atypical MSA cases

	Horoupian et al. [13]	Shibuya et al. [32]	Piao et al. [31]	Konagaya et al. [19]	Katayama et al. [16]	Yuasa et al. [46]
<b>Clinical features</b>						
Nationality	US	JPN	JPN	JPN	JPN	JPN
Sex	Female	Female	Female	Male	Female	Female
Age at death, years	78	65	73	66	70	77
Age at onset, years	75	53	54	51	45	63
Duration of illness, years	3	12	19	15	25	14
Clinical diagnosis	Parkinsonism plus	MSA	MSA	MSA	n.a.	SCD
Cerebellar signs	+	+	+	+	+	+
Parkinsonism	+	+	+	+	+	
Memory impairment		+	+	+		+
<b>Pathologic features</b>						
Brain weight, grams	1140	920	687	1115	n.a.	1065
Cerebral atrophy	F	T	F, T	F, T	T	T
SND/OPCA	SND > OPCA	SND < OPCA	SND = OPCA	SND = OPCA	SND < OPCA	SND < OPCA
Numerous GCI	Yes	Yes	Yes	Yes	Yes	Yes
Many limbic NCI	Yes	Yes	Yes	Yes	Yes	Yes
Morphology	PiB-like	PiB-like	PiB-like	n.a.	PiB-like	n.a.
$\alpha$ -Synuclein stain	n.a.	n.a.	Positive	Positive	Positive	Positive
Silver stain	Positive	Positive	Positive	Positive	Positive	Positive
Tau stain	Negative	Negative	Positive (partially)	Negative	Negative	Negative
AD-type pathology	Minimal	Minimal	Minimal	Minimal	n.a.	Minimal

*F* frontal lobe, *T* temporal lobe, *SND* striatonigral degeneration, *OPCA* olivopontocerebellar degeneration, *SCD* spinocerebellar degeneration, *US* United states, *JPN* Japan, *n.a.* not available, *PiB-like* Pick body-like

atypical MSA—FTLD-synuclein. Alternatively, atypical MSA could be considered a subtype of MSA in addition to MSA-P and MSA-C, namely MSA-FTD. From a clinical perspective, this is a more challenging diagnosis, since none of our patients carried an antemortem diagnosis of MSA; rather, the clinical picture was that of FTD.

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