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CORRESPONDENCE

Screening for α-synuclein immunoreactive neuronal inclusions in the hippocampus allows identification of atypical MSA (FTLD-synuclein)

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Atypical multiple system atrophy (aMSA) is a term recently introduced by Aoki et al. to describe cases that show hallmark neuropathological changes of glial cytoplasmic inclusions (Papp–Lantos bodies) characteristic of MSA, while clinically presenting with frontotemporal dementia (FTD) syndromes associated with frontotemporal lobar degeneration (FTLD) and severe limbic and cortical α-synuclein neuronal pathology [2]. The authors evaluated FTD syndrome cases and showed that the evaluation of α-synuclein immunoreactive neuronal cytoplasmic inclusions (NCIs) in the hippocampus (dentate gyrus and CA1/Subiculum) seems to be of great importance [2]. We aimed to determine if these morphological features in the hippocampus are reliable to identify similar cases in our archives.

We evaluated α-synuclein immunostaining in the hippocampus from a cohort of 18 neuropathologically confirmed MSA cases [10]: all cases contained characteristic Papp–Lantos bodies and NCIs in the basal ganglia, brainstem, and cerebellum. According to Aoki et al. [2], we used a four-tiered scoring system (none—0, mild—1, moderate—2, severe—3) for the evaluation of α-synuclein NCIs regardless of their morphology (ring, NFT, or Pick body like). The selection was blinded to the clinical diagnosis, age, gender, and macroscopic observations. We observed α-synuclein immunoreactive NCIs in the granule cells of the dentate gyrus in seven cases (38 %). Five out of seven cases (online supplemental file 1) showed only relatively few NCIs in the dentate gyrus and CA1/Subiculum (score 1) (Fig. 1a, b). There was a lack of α-synuclein immunoreactive thin neurites and eosinophilic Pick body-like spherical inclusions in the hematoxylin and eosin (H&E) staining. Three of these five cases did not show clinical symptoms of dementia. Gait disturbance, parkinsonism, cerebellar symptoms, and dementia (not compatible with FTD) were reported in the two additional cases (57- and 71-year-old women) during the final 24 months of illness (total duration of illness was 120 and 75 months, respectively). Both brains showed Aβ plaques (both Thal phase 3) [9] and neurofibrillary degeneration (both Braak stage II) [1, 3]. In the

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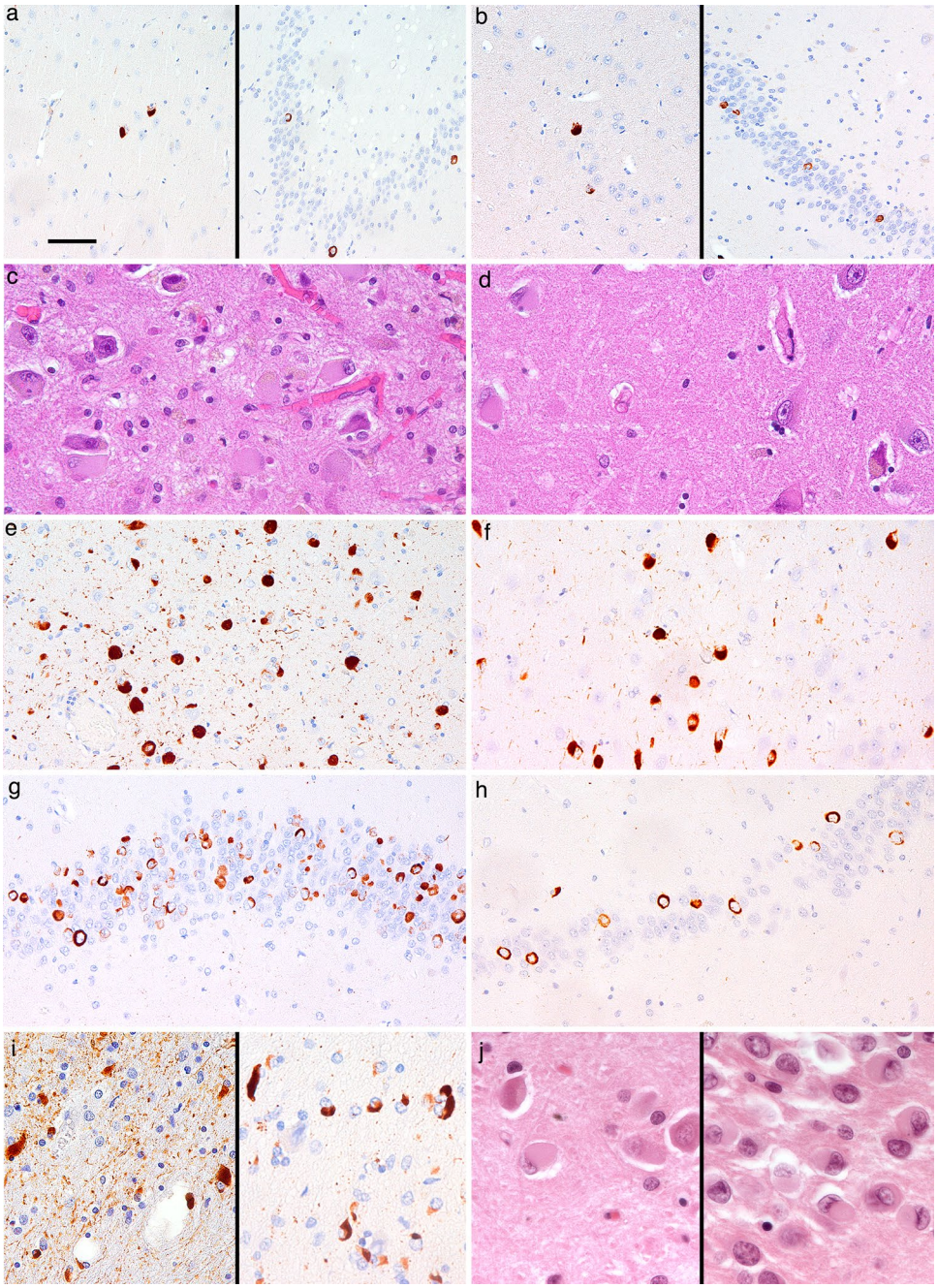

Fig. 1 Microscopic findings in the reported atypical MSA cases. Few spherical [*left side* of images (**a**) and (**b**); CA1 subregion] and ring-shaped [*right side* of images (**a**) and (**b**); dentate gyrus] neuronal α-synuclein immunoreactive inclusions in two cases with typical MSA and dementia in the last phase of the disease course. Spherical eosinophilic inclusions in atypical MSA (FTLD-synuclein) in the CA1 subregion (**c** case 1; **d** case 2). Many Pick body-like and NFT-like inclusions in the CA1/Subiculum in case 1 (**e**) and 2 (**f**). α-Synuclein immunoreactive ring-shaped inclusions in the granule cells of the dentate gyrus in case 1 (**g**) and 2 (**h**). Immunostaining for phospho-tau (AT8 antibody) reveals oligodendroglial inclusions in the hippocampal white matter partly with globular morphology (**i** *left side* of image) resembling α-synuclein immunoreactive Papp–Lantos bodies (**i** *right side* of image). Spherical eosinophilic inclusions in atypical MSA (FTLD-synuclein) reported by Sikorska et al. [8] (**j** *left side* of image shows CA1 and *right side* of image shows dentate gyrus). The *black bar* in the *bottom* of image (**a**) represents a length of 100 μm for images **a** and **b**; 30 μm for images **c**, **d** and **j**; and 50 μm for images **e**–**i**

cases lacking hippocampal NCIs, FTD-like symptoms have not been reported.

An additional two cases showed severe NCI pathology in the hippocampus, both in the dentate gyrus and the CA1/Subiculum. NCIs in the dentate gyrus mostly had ring-shaped immunoreactivity. Aoki and colleagues considered numerous Pick body-like inclusions in the hippocampus to be highly characteristic of aMSA [2]; indeed, we also observed many of these together with NFT-like inclusions [2] (Fig. 1c–h) and therefore interpreted these cases as aMSA. Evaluation of the clinical data revealed FTD syndromes in these two cases, associated with macroscopic signs of FTLD. Patient 1 (72-year-old man) presented with parkinsonism, and soon developed dysarthria, dysphagia, vertical gaze palsy and FTD symptoms (i.e., utilization behavior and frontal lobe symptoms) during the disease course, which lasted 42 months (online supplemental file 1). The clinical diagnosis was progressive supranuclear palsy with frontal dementia. The second patient (79-year-old woman) presented with non-fluent aphasia, impaired word finding, and subsequent memory disturbance associated with gait disorder and parkinsonism, which rapidly

progressed and was accompanied by akinetic mutism in the terminal phase. EEG revealed transient left temporal sharp wave complexes. Since the disease course was shorter than 18 months and the laboratory examinations did not suggest an alternative cause for the symptoms, a tentative diagnosis of Creutzfeldt–Jakob disease was rendered. Neither of the two cases had documented autonomic dysfunction.

In the cohort of Aoki et al. [2], hippocampal tau pathology spanned from mild to severe NFT pathology with variable Aβ pathology. These pathologies were not significantly different from typical MSA cases [2]. In our two aMSA cases, we observed moderate neurofibrillary degeneration (Braak III and II) and also Aβ deposits (online supplemental file 1). It is noteworthy, however, that both cases showed oligodendroglial AT8 immunopositive inclusions only in the hippocampal white matter, despite the lack of argyrophilic or p62-positive grains. Although some of these showed globular morphology (Fig. 1i), the amount and their anatomically restricted distribution was not compatible with that reported in globular glial tauopathies [5].

As the next step, we re-evaluated the case reported by Sikorska et al. [8]. Indeed, the early clinical symptoms of this individual included behavioral changes followed by psychotic symptoms, with aggression and later delusion and hallucinations followed by rapidly progressive dementia. Although not covered by current diagnostic criteria, psychotic symptoms seem to be common in FTD patients [6]. This clinical phenotype together with the prominent degree of FTLD and abundant presence of NCIs (including NFT, Pick body, and ring like) in the hippocampus and dentate gyrus (Fig. 1j) allows this case to be diagnosed as aMSA (FTLD-synuclein). In that paper [8], cortical Lewy bodies were described in the cortex, which strongly resemble the Pick body-like inclusions reported by Aoki et al. [2]. Indeed, the archival diagnosis was Pick's disease [8]. It is worth noting that the clinical phenotype of the cases considered to be FTLD-synuclein (see Table 4 in the paper by Aoki et al. [2]) was classified based on the presence of FTLD and an abundance of limbic and cortical NCIs, although the clinical symptoms were not fully compatible with FTD.

Based on these observations, we have expanded the series reported by Aoki et al. [2] by reporting two previously unpublished cases. Additionally, we can confirm that the case reported by Sikorska et al. [8] also belongs to the group of aMSA. All three cases have shorter illness durations than those reported in Japan and bear similarity to those reported in the USA [2]. We agree that these cases show either or both features of FTLD or clinical symptoms of FTD. In addition, we have also observed that the clinical progression can suddenly become quite rapid, which can introduce a differential diagnosis of prion disease. Our findings support the suggestion that diagnostic screening of the hippocampus for neurodegeneration-related proteins can reliably suggest a neurodegenerative condition [4, 7]. Accordingly, detection of a moderate or severe degree of eosinophilic and α-synuclein immunoreactive NCIs with NFT-, or Pick body-like appearance in the CA1/subiculum and ring-like or spherical NCIs in the granule cells of the dentate gyrus and α-synuclein immunoreactive thin neurites and reactive astrogliosis strongly suggests aMSA (FTLD-synuclein) warranting a systematic evaluation.

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Compliance with ethical standards

Conflict of interest The authors report no conflict of interest.

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Online Supplemental file 1. Clinical and neuropathological features of MSA cases with hippocampal neuronal α-synuclein immunoreactive inclusions. aMSA indicates cases classified as atypical MSA (FTLD-synuclein) and "Hippocampus NCI Case" indicates cases that showed low amount of hippocampal NCIs and were not classifed as atypical MSA. (−) indicates “no” and (+) indicates “yes” (present). BGG: basal ganglia; Cbll: cerebellum; GCI: glial cytoplasmic inclusions; NCI: neuronal cytoplasmic inclusion; WM: white matter.

Variable	aMSA Case 1	aMSA Case 2	Hippocampus NCI Case 1	Hippocampus NCI Case 2	Hippocampus NCI Case 3	Hippocampus NCI Case 4	Hippocampus NCI Case 5
Age	72	79	71	57	63	54	48
Sex	male	female	female	female	male	male	female
Duration of illness (years)	3.5	1.5	6.25	10	6	4	6
Initial symptoms	Rigidity and bradykinesia	Aphasia and gait disorder	Gait disturbance, dizziness	Falls, Rigidity and bradykinesia	Rigidity and bradykinesia	Rigidity and bradykinesia	Rigidity and bradykinesia
Overview of symptoms							
Aphasia	−	+	−	−	−	−	−
Parkinsonism	+	+	+	+	+	+	+
Dysarthria	+	−	+	−	+	+	+
Dysphagia	+	−	+	−	−	−	+
Cerebellar signs	-	+	+	+	+	−	+
Myoclonus	−	+	−	−	−	−	−
Early memory impairment	+	+	−	−	−	−	−
Cognitive decline in late stage of disease	+	+	+	+	−	−	−
Pyramidal signs	−	−	+	−	−	−	+
Utilization Behavior	+	−	−	−	−	−	−
Gaze palsy	+	−	−	−	−	−	−
Orthostatic hypotension	−	−	+	+	−	−	−
Clinical diagnosis	PSP-FTD	CJD	Parkinson syndrome + Dementia	Parkinson syndrome + Dementia	MSA-P	Parkinson syndrome	MSA-P
Cerebral atrophy	Frontotemporal and Striatal	Frontotemporal and Striatal	Striatal and cerebellar	Suppl. Motor Area, Cerebellar	Brainstem	Brainstem	Striatal, brainstem, cerebellar
Braak NFT stage	III	II	II	II	I	0/Ia (single NFT in pons)	0
Thal phase	4	3	3	3	0	0	3
TDP-43 pathology	−	−	−	−	−	−	−
GCIs in Cbll, BGG, Brainstem	+	+	+	+	+	+	+
Neuronal loss/Gliosis (score)							
Dentate gyrus	2	2	0	0	0	0	0
CA1/subiculum	2	2	0	0	0	0	0
Eosinophilic spherical NCIs (score)	2	2	0	0	0	0	0
α-synuclein NCIs (score)							
Dentate gyrus	3	3	1	1	1	1	1
CA1-4/Subiculum	3	3	1	1	1	1	1
Temporal Cx	2	2	1	1	0	0	0
Frontal Cx	2	1	1	1	0	0	0
α-synuclein NT-CA1/subiculum (score)	3	2	0	0	0	0	0
α-synuclein GCIs (score)							
Frontal WM	2	0	2	3	0	0	2
Temporal WM	3	1	2	2	2	2	2
Hippocampus (Alveus)	3	2	2	3	2	2	1