Hippocampal α-synuclein pathology correlates with memory impairment in multiple system atrophy

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Abstract

Recent postmortem studies reported 22 to 37% of patients with multiple system atrophy (MSA) can develop cognitive impairment. With the aim of identifying associations between cognitive impairment including memory impairment and α-synuclein pathology, 148 consecutive patients with pathologically proven MSA were reviewed. Among them, 118 (79.7%) were reported to have had normal cognition in life, whereas the remaining 30 (20.3%) developed cognitive impairment. Twelve of them had pure frontal-subcortical dysfunction, defined as the presence of executive dysfunction, impaired processing speed, personality change, disinhibition or stereotypy; 6 had pure memory impairment; and 12 had both types of impairment. Semi-quantitative analysis of neuronal cytoplasmic inclusions in the hippocampus and parahippocampus revealed a disease duration-related increase in neuronal cytoplasmic inclusions in the dentate gyrus and cornu ammonis regions 1 and 2 of patients with normal cognition. In contrast, such a correlation with disease duration was not found in patients with cognitive impairment. Compared to the patients with normal cognition, patients with memory impairment (pure memory impairment: N=6; memory impairment + frontal-subcortical dysfunction: N=12) had more neuronal cytoplasmic inclusions in the dentate gyrus, cornu ammonis regions 1-4 and entorhinal cortex. In the MSA mixed pathological subgroup, which equally affects the striatonigral and olivopontocerebellar systems, patients with the same combination of memory impairment developed more neuronal inclusions in the dentate gyrus, cornu ammonis regions 1, 2 and 4, and the subiculum compared to patients with normal cognition. Using patients with normal cognition (N=18), frontal-subcortical dysfunction (N=12) and memory impairment/memory impairment + frontal-subcortical dysfunction (N=18), we further investigated whether neuronal or glial cytoplasmic inclusions in the prefrontal, temporal and cingulate cortices or the underlying white matter might affect cognitive impairment in patients with MSA. We also examined topographic correlates of

frontal-subcortical dysfunction with other clinical symptoms. Although no differences in neuronal or glial cytoplasmic inclusions were identified between the groups in the regions examined, frontal release signs were found more commonly when patients developed frontal-subcortical dysfunction, indicating the involvement of the frontal–subcortical circuit in the pathogenesis of frontal-subcortical dysfunction. Here, investigating cognitive impairment in the largest number of pathologically proven MSA cases described to date, we provide evidence that neuronal cytoplasmic inclusion burden in the hippocampus and parahippocampus is associated with the occurrence of memory impairment in MSA. Further investigation is necessary to identify the underlying pathological basis of frontal-subcortical dysfunction in MSA.

Introduction

Multiple system atrophy (MSA) is an adult-onset, sporadic, fatal neurodegenerative disorder clinically characterized by the presence of autonomic dysfunction, together with poorly levodopa-responsive parkinsonism and/or cerebellar ataxia (Déjerine and Thomas, 1900; Shy and Drager 1960; Adams *et al*., 1961; Graham *et al*., 1969; Quinn. 1989). Neuropathological features in MSA include the widespread occurrence of α-synuclein-immunopositive glial cytoplasmic inclusions (GCIs) with, to a lesser extent, neuronal cytoplasmic inclsions (NCIs), neuronal nuclear inclusions and glial neuronal inclusions (Papp *et al*., 1989; Kato *et al.,* 1990; Nakazato *et al*., 1990; Papp *et al*., 1992; Ozawa *et al.,* 2004). MSA is now classified into two clinical subtypes, based on the predominant motor presentation: a parkinsonian variant (MSA-P) related to striatonigral degeneration (SND) and a cerebellar variant (MSA-C) reflecting olivopontocerebellar atrophy (OPCA) (Fanciulli *et al*., 2015). Although neuronal loss and α-synuclein pathology are widely distributed and are not limited to either the SND or OPCA system at autopsy, these two clinical subtypes are the reflection of the anatomical systems predominantly involved in neurodegeneration during the disease process of MSA. Previously, we reported that 34% of 100 pathologically proven MSA cases were SND predominant (MSA-SND); 17% were OPCA predominant (MSA-OPCA); and the remaining 49% had the equal involvement of SND and OPCA systems (MSA-mixed) (Ozawa *et al*., 2004).

Different from the core features of MSA including autonomic dysfunction, parkinsonism or cerebellar ataxia, cognitive impairment in MSA is clinically heterogeneous and its neuropathological substrate remains uncertain. In addition, in contrast to increased awareness of cognitive impairment in Parkinson's disease (PD) (Green et al., 2002; Aarsland and Kurz, 2010), cognitive impairment has been underestimated and under-investigated as a clinical feature of MSA. Although the estimated prevalence of cognitive impairment in patients

MSA varies depending on retrospective postmortem studies with different settings, 22 to 37% of patients with pathologically proven MSA were reported to have developed some degree of cognitive impairment during the course of their illness (Wenning *et al*., 1997; Cykowski *et al.,* 2015; Koga *et al*., 2015; Koga *et al*., 2017). Executive function is most commonly compromised in patients with MSA, followed by memory and information processing speed (Stankovic *et al*., 2014; Fiorenzato *et al*., 2017; Koga *et al*., 2018). Aoki et al. also reported four patients with pathologically proven MSA with predominant NCIs in medial temporal lobe and limbic structures who had presented clinically with frontotemporal dementia (Aoki et al., 2015). With the aim of identifying associations between cognitive impairment and α-synuclein pathology, our group previously investigated MSA cases with or without cognitive impairment. However, no difference was found in GCI and NCI burden in the cortical and limbic regions between the two groups (Asi *et al*., 2014). On the other hand, Cykowski et al. have revealed that the presence of globular NCIs in the neocortex was associated with cognitive impairment (Cykowski *et al.,* 2015). In contrast, Koga et al. have demonstrated that 33 (32%) of 102 autopsy-proven MSA patients developed some degree of cognitive impairment and had a greater burden of NCIs in the dentate gyrus rather than the neocortex, compared to those without cognitive impairment (Koga *et al*., 2017). These inconsistent findings have prompted us to reappraise cognitive impairment and its related pathologies in MSA.

In the present study, 148 cases with pathologically proven MSA were examined to investigate the pathology underlying cognitive impairment. We hypothesised that memory impairment (MI) and frontal-subcortical dysfunction (FSD), including executive dysfunction and impaired processing speed, can be attributed to different pathological substrates. We therefore investigated NCIs and GCIs in neocortical and limbic regions in pathologically proven MSA cases with MI, FSD and normal cognition (NC).

Materials and Methods

Patients

We identified 158 consecutive patients with a neuropathological diagnosis of MSA from the archive of the Queen Square Brain Bank for Neurological Disorders (QSBB) between 2002 and 2018. The brain donation program and protocols have received ethical approval for donation and research by the NRES Committee London – Central, and tissue is stored for research under a license issued by the Human Tissue Authority (No. 12198).

Medical record review

We systematically reviewed all available medical records for the 158 MSA patients. There is overlap with the QSBB cohort recently reported (Miki *et al*., 2019). The primary care medical records, correspondence between medical specialists and general practitioners, National Hospital for Neurology and Neurosurgery medical files, and the QSBB self-assessment data were utilised for the study. All patients had been examined by experienced hospital specialists (consultant physicians, geriatricians, general neurologists, movement disorder specialists) during the course of their illness. Information from the case notes was extracted by one neurologist (Y. M). A final study cohort of 148 cases remained after the exclusion of 10 cases due to: inadequate medical records (3 cases); severe autonomic neuropathy due to other causes including diabetic autonomic neuropathy, which might affect diagnostic accuracy of MSA (5 cases); severe tissue artefact potentially affecting semi-quantitative study (1 case); deep brain stimulation, which might cause cognitive and psychiatric impairment related to the procedure (1 case) (Parsons *et al.,* 2006; Appleby *et al*., 2007).

Clinical features evaluated in the present study were: i) age of onset: age, in years, when the first symptom considered to be attributable to the neurological disorder was reported; ii) age at death; iii) disease duration: time between the age of onset and the age at death; iv) diagnostic accuracy of MSA; v) family history: recorded as present if a first- or second-degree family history of neurodegenerative disease was documented; vi) onset of cognitive

impairment: time between the age of onset and the time when cognitive impairment was first documented by a clinician; vii) FSD including executive dysfunction, impaired processing speed, personality change, disinhibition and stereotypy; viii) MI; ix) frontal release signs: defined as presence of at least one of the following signs: Gegenhalten, snout reflex, palmomental reflex or grasp reflex; x) depression; xi) hallucinations; xii) REM sleep behavior disorder: recorded as present if confirmed on polysomnography or if it was clinically suspected based on the behavioral description by the bed partner; xiii) urinary incontinence; and xiv) orthostatic hypotension: defined as a > 30 mm Hg systolic or 15 mm Hg diastolic blood pressure drop on standing, or repeated episodes of syncope.

Definition of cognitive impairment

Cognitive impairment was considered as present if executive dysfunction, impaired processing speed, MI, personality change, disinhibition or stereotypy was documented by a clinician or confirmed by a neuropsychological test (the Wechsler Adult Intelligence Scale (WAIS) -R or III). MMSE, Addenbrooke's Cognitive Examination-Revised or III was also used to evaluate the presence of cognitive impairment. Executive dysfunction and impaired processing speed, which can arise from the disruption of frontal-subcortical circuits, were categorized as FSD (Stankovic *et al*., 2014; Fiorenzato *et al*., 2017). Because some patients with MSA are known to develop frontotemporal dementia, personality change, disinhibition and stereotypy were also categorized as FSD in the present study (Aoki *et al*., 2015; Rohan *et al*., 2015). As WAIS-III was performed in only one patient with NC, verbal or performance IQ was not included for comparisons of patients with or without cognitive impairment. Two patients who had significant cognitive impairment documented, but with no further detailed documentation in their clinical records were classified as FSD subgroup. Patients' subjective complaints or caregivers' impressions were not included in this study. In addition, we also

avoided speculating about the presence of cognitive impairment based on individual's activities of daily living (ADL) because severe motor dysfunctions including marked dysphonia or dysarthria can make it difficult to assess mild cognitive impairment especially in the advanced stage of disease.

Neuropathological methods

The brains were fixed with 10% buffered formalin for around three weeks. For immunohistochemical examinations, eight-μm-thick, formalin-fixed, paraffin-embedded sections from the prefrontal cortex and white matter (superior and middle frontal gyri), temporal cortex and white matter (superior and middle temporal gyri), cingulate cortex and bundle, amygdala, hippocampus and parahippocampus were immunostained with mouse monoclonal antibodies against amyloid β (M0872; Dako, Ely, UK; 1:100), α-synuclein (MA1-90342; Thermo Scientific, Waltham, MA; 1:1,500) and tau (MN1020; Thermo Scientific; 1:600). Based on the degree of neuronal cell loss, MSA was classified into MSA-SND, MSA-OPCA and MSA-mixed subtypes based on previously published criteria (Ozawa *et al*., 2004). Because it can be difficult to distinguish cortical Lewy bodies from NCIs with α-synuclein immunohistochemistry, concomitant Lewy bodies were determined in the substantia nigra and the locus coeruleus using hematoxylin and eosin staining. Alzheimer pathology, neuritic amyloid β plaques and neurofibrillary tangles (NFTs), were evaluated according to the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) scheme and Braak NFT stage, respectively (Alafuzoff *et al*., 2008; Hyman *et al*., 2012).

Semi-quantitative analysis

NCI burden was assessed using α-synuclein immunohistochemistry in 11 brain regions: the hippocampus and parahippocampus (dentate gyrus, cornu ammonis (CA) regions 1-4,

subiculum - including pre- and peri-subiculum - and entorhinal cortex), amygdala, and the prefrontal, temporal and cingulate cortices. NCIs were identified based on the features of the nucleus. For semi-quantitative analyses, we used a four-point scale, the same as the approach described by Koga et al: 0: absent; 1+: mild; 2+: moderate; and 3+: severe (Fig. 1A-I) (Koga *et al*., 2017). Previous studies have shown no difference in GCI load in the neocortex and limbic regions including the hippocampus and amygdala in cognitively impaired MSA patients (Asi *et al*., 2014; Koga *et al*., 2017). Therefore, in the present study, the density of GCIs in the prefrontal and temporal white matter and cingulate bundle was assessed using a modified grading scale as described previously: 0: 0-5 inclusions; 1+: 6-20 inclusion; 2+; 21-40 inclusions; 3+; ≥41 inclusions (Fig. 1J-L) (Ozawa *et al*., 2004). The density of NCIs or GCIs was evaluated using a x20 objective, and the most affected field in each region investigated was chosen as a representative area for semi-quantitative analysis.

Statistical analysis

All statistical analyses in the present study were performed using SPSS 25.0 (SPSS Inc,USA) or R commander (Rcmdr 1.3-5 package, R2.8.1). The Chi-square or Fisher's exact tests with Bonferroni correction was performed for categorical variables. After Shapiro-Wilk test, a test of normality, one-way ANOVA followed by Tukey test for parametric data or Kruskal-Wallis test followed by Steel-Dwass test for non-parametric data was performed for continuous variables. Spearman's rank correlation was used to evaluate correlations. A probability value of less than 0.05 ($P < 0.05$) was considered to be significant.

Data availability

The raw data that support the findings of the present study are available on request from the corresponding author.

Results

Clinical characteristics

Demographic and clinical characteristics of MSA patients with NC, FSD and MI are shown in Table 1. The vast majority (98.6%) of the patients were reviewed at least once by neurologists during the course of their illness. Of 148 patients with pathologically proven MSA, 118 (79.7%) were reported to have had normal cognition during the course of their illness, whereas the remaining 30 (20.3%) developed documented cognitive impairment. The subtypes of cognitive impairment are outlined as follows: 12 (8.1%) had pure FSD; 6 (4.1%) presented with pure MI; and 12 (8.1%) developed both MI and FSD. Of all the 30 patients with cognitive impairment, 18 (60%) received either mini-mental state exam (MMSE), Addenbrooke's Cognitive Examination-Revised or neuropsychological evaluations (WAIS-III or R) during the course of their illness, whereas only 12 (11.1%) of 118 patients with NC had either of these assessments (MMSE, abbreviated MMSE or Addenbrooke's Cognitive Examination III in 11 cases; WAIS-III in 1 case). Cognitive tests were performed at variable intervals before death. There was no statistical difference in age of onset, age at death, disease duration, diagnostic accuracy, onset of cognitive impairment, depression, hallucination, REM sleep behavior disorder and urinary incontinence among any combinations of clinical subgroups. MMSE could not be compared among clinical subtypes due to the small number of patients with pure MI that underwent the exam (N = 1). Comparative studies on cognitive impairment between MSA-P and MSA-C have been reported with heterogeneous results (Kawai *et al.,* 2008; Chang *et al.,* 2009). In the present study, although it did not reach statistical significance, 77.8% (N = 7/9) of patients with pure FSD were MSA-C. Additionally, it has been reported that orthostatic hypotension can be associated with the occurrence of cognitive impairment in MSA (Udow *et al.,* 2016); however, no correlation was found between them. Clinical features of each patient

with cognitive impairment are shown in Table 2. Among 30 patients with cognitive impairment, 19 had executive dysfunction, 18 had MI, 7 had impaired processing speed, 6 had personality change, and 2 had disinhibition. Five (16.7%) of 30 patients with cognitive impairment were reported to have had documented dementia, which met the criteria defined by ICD-10. Cognitive functions were moderately to severely affected in case 27 and 30. In particular, case 30 first developed MI at the age of 57, followed by nominal dysphasia, executive dysfunction and personality change in addition to asymmetric left sided parkinsonism and autonomic dysfunctions. Cognitive impairment was predominant during the course of her illness. The patient was treated with Rivastigmine. While the severity of cognitive impairment was not clearly described, two patients (case 21 and 23) required typical or atypical antipsychotics to control their psychosis. MSA patients who developed FSD in life (pure FSD: N = 3/12; MI+FSD: N = 4/12) had frontal release signs more frequently than patients with NC (NC: N = 10/118) (NC vs FSD/MI+FSD: 8.5% vs 29. 2%; $P < 0.05$).

Pathological characteristics

There was no difference in pathological features including brain weight, pathological subtype, Braak NFT stage and CERAD plaque score and concomitant Lewy bodies among any combinations of clinical subgroups (Table1). Skrobot et al. evaluated the risk of cerebral vascular disease contributing to cognitive impairment with the combination of three determinants: large (> 10 mm) subcortical cerebral infarcts, moderate to severe occipital leptomeningeal cerebral amyloid angiopathy, and moderate to severe occipital white matter arteriolosclerosis. These determinants were used to assign a low, moderate or high risk of vascular cognitive impairment (Skrobot *et al.,* 2016). In the present study, only four (3.7%) of 118 patients with NC had a moderate risk of cognitive impairment due to cerebrovascular disease, whereas no patients with cognitive impairment had a moderate or severe risk (Table1).

The neuropathological examination of case 30, which clinically developed severe dementia including MI and FSD, revealed slight frontal lobe atrophy and moderate atrophy of the temporal lobe, which was most apparent in the anterior temporal lobe and involved the medial temporal lobe structures (supplementary Fig. 1A, B). Microscopically, severe neuronal loss was noted in the hippocampus and parahippocampal and fusiform gyri. α-Synuclein immunohistochemistry revealed numerous ring-shaped NCIs in a majority of the granule cells of the dentate fascia (Supplementary Fig. 1C) and NFT-like NCIs in the remaining neurons of CA1 and CA4 (Supplementary Fig. 1D). In addition, a number of NCIs were seen in superficial as well as deep layers of the frontal and temporal lobes (Supplementary Fig. 1E). There was atrophy of the lateral putamen with neuronal loss. Both the substantia nigra and locus coeruleus demonstrated severe depletion of their pigmented neurons. GCIs were widely distributed in the brain. Based on these pathological findings, case 30 was considered to be an example of frontotemporal lobar degeneration-synuclein (Aoki *et al*., 2015; Rohan *et al*., 2015).

NCIs in the hippocampus and parahippocampus

Several types of NCIs were found in the hippocampus and parahippocampus. In the dentate gyrus, ring-shaped NCIs were particularly frequent (Fig. 2A) and, less commonly, Pick body-like NCIs were also found (Fig. 2B), while in CA1-4, subiculum and entorhinal cortex, NFT-like NCIs were frequently found (Fig. 2C).

First, we aimed to study the relationship between MI and NCI burden in the hippocampus and parahippocampus. For this purpose, patients with MI with (MI+FSD: N = 12) or without FSD (Pure MI: N = 6) were analysed together and compared with patients with pure FSD (N = 12). We have previously reported that NCIs in SND or OPCA system were not associated with disease duration (Ozawa *et al.,* 2004). On the other hand, Brettschneider et al. demonstrated that patients had NCIs in CA1 or CA2 seven years after their onset, suggesting

that NCIs can increase with disease duration at least in some regions, including the hippocampus (Brettschneider *et al.*, 2018). Thus, we assessed NCIs in the hippocampus and parahippocampus in MSA-NC, showing a weak but significant correlation between disease duration and the number of NCIs in the dentate gyrus (r_s = 0.29; P = 0.01), CA1 (r_s = 0.22; P = 0.02) and CA2 (r_s = 0.02; P = 0.02) (Spearman's rank coefficient) (Supplementary Table 1). We further examined the association of the number of NCIs with disease duration depending on pathological subtypes. Interestingly, MSA-mixed pathological subtype of MSA-NC revealed a strong correlation between NCI load and disease duration in the same regions described above (dentate gyrus: r_s = 0.6, P = 0.00006; CA1: r_s = 0.4, P = 0.013; CA2: r_s = 0.5, P = 0.01) (Spearman's rank coefficient) (Supplementary Table 1). In contrast, no correlation between NCI load and disease duration was observed in patients who developed MI in life (MI/MI+FSD subgroup) (data not shown). ~~Subtype analysis showed that only MSA-OPCA pathological subtype of MSA-MC had a correlation (r_s = 0.67, P = 0.024).~~ Next, we examined NCI burden between MSA-NC, MSA-FSD and MSA-MI/MI-FSD. Compared to patients with NC, patients with MI/MI+FSD had more NCIs in the dentate gyrus, CA1, CA2, CA3 and CA4 and entorhinal cortex (Fig. 3A-G). In addition, more NCIs were found in the CA4 of patients with MI/MI+FSD than that of patients with FSD (Fig. 3E). NCI burden was then assessed based on the pathological subtypes. In the MSA-mixed pathological subgroup, patients with MI/MI+FSD had more NCIs than patients with NC in the dentate gyrus, CA1, CA2, CA4 and subiculum (Fig. 4A-C, E and F). Patients with pure MI also had a greater burden of NCIs in the dentate, CA2 and CA4, suggesting that FSD is not underpinned by NCI load in the hippocampus and parahippocampus (Supplementary Fig. 2A-G). Furthermore, the MSA mixed pathological subgroup of patients with MI also had similar results to those of patients with MI/MI+FSD (Supplementary Fig. 3A-G). Thus, NCIs in some hippocampal subfields can be associated with disease duration in patients without cognitive impairment. In contrast, MSA

patients with MI/MI+FSD, irrespective of disease duration, can have more NCIs in widespread regions of the hippocampus and parahippocampus when compared to patients with NC.

NCIs in the neocortex, cingulate cortex and amygdala

Next, we examined whether NCI load in the prefrontal, temporal and cingulate cortices, and the amygdala is associated with MI or FSD in patients with MSA. Representative examples of NCIs in the prefrontal, temporal and cingulate cortices and amygdala are shown in Fig. 2D-F. For this investigation, we selected 18 patients with NC matched for gender, age of death, disease duration and pathological subtypes (Male: N = 9, 50%; age of onset: 65.7 ± 6.7; disease duration 8.4 ± 2.8; MSA-SND: N = 3, 16.7%; MSA-OPCA: N = 11, 61.1%; MSA-mixed; N = 4, 22.2%). NCIs in each region were then assessed between patients with NC (N = 18), FSD (N = 12) and MI/MI+FSD (MI: N = 6; MI+FSD: N = 12). There was no difference in NCI burden between these three clinical subgroups (Fig. 5A-D). Even when patients with MI+FSD cases were incorporated into the FSD subgroup (FSD/MI+FSD), no difference in NCI load was found between patients with NC, FSD/FSD+MI, and MI (data not shown).

GCIs in the white matter of the prefrontal, temporal and cingulate regions

Finally, we studied GCI load in the subcortical white matter of the prefrontal and temporal lobes and also the cingulate bundle between patients with NC (N = 18), FSD (N = 12) and MI/MI+FSD (MI: N = 6; MI+FSD: N = 12). No statistical difference was found in the GCI burden in these white matter regions between the three clinical subgroups (Fig. 6A-C). When patients MI+FSD cases were incorporated into the FSD subgroup, no difference was found in GCI burden between the three clinical subgroups, indicating that the density of GCIs in these regions is unlikely to be associated with the occurrence of cognitive impairment (data not shown).

Discussion

Previous studies have failed to identify the pathological substrates of MI in MSA. This may be because cognitive impairment in MSA was evaluated as a single entity, making each underlying pathology of cognitive impairment, including FSD or MI, less discernible (Wenning et al., 1997; Asi *et al*., 2014; Cykowski *et al*., 2015; Koga *et al*., 2017). We have investigated the largest number of pathologically proven MSA cases described for such a study to date. By assessing the pathological substrates of cognitive impairment depending on predicted regions: the frontal-subcortical circuit for FSD and the hippocampus for MI, we have demonstrated that NCIs in the hippocampus and parahippocampus are associated with MI in MSA. We found that patients with MI/MI+FSD had more NCIs in widespread regions of the hippocampus and parahippocampus than patients with NC. Among the NC group, NCI load in the dentate gyrus, CA1 and CA2 increased with the disease duration. Such a correlation between NCI load and disease duration was not observed in patients who developed MI in life (MI/MI+FSD subgroup). In addition, subgroup analysis of MSA pathological subtype further demonstrated a greater NCI load in hippocampus and parahippocampus in patients with MI/MI+FSD than patients with NC. These findings may suggest other inherent factors that play a role in an accelerated NCI formation in these regions, which in turn results in cognitive impairment.

It has been reported that accumulation of abnormal proteins in the hippocampus and parahippocampus contributes to the occurrence of MI in neurodegenerative disorders including Alzheimer's dementia (AD) and Lewy body diseases (PD or dementia with Lewy bodies) (Gómez-Isla *et al.,* 1996; Gómez-Isla *et al.,* 1997; Flores-Cuadrado *et al.,* 2016; Adamowicz *et al.,* 2017). In AD, in which MI predominantly develops from the early disease stage, the severity of MI was associated with the number of NFTs, which increase with disease duration in the hippocampus and parahippocampus. More importantly, neuronal loss exceeded that of

NFT, showing even better correlation with cognitive decline in AD (Gómez-Isla *et al.,* 1996; Gómez-Isla *et al.,* 1997). In the present study, similar to AD, widespread α-synuclein pathology was found in the hippocampus and parahippocampus. However, no correlation between disease duration and severity of α-synulcein pathology was found in our cohort. In addition, there was no, or at least only mild, neuronal loss in the hippocampus and parahippocampus of MSA patients with cognitive impairment, except for case 30. On the other hand, in PD, α-synulcien pathology in CA2 increased along with pathological Braak PD stage (Braak *et al.,* 2003; Flores-Cuadrado *et al.,* 2016). In dementia with Lewy bodies, Lewy bodies in EC and intraneuritic Lewy bodies in CA2 were the cardinal features in the hippocampus and parahippocampus; however, there was very mild α-synuclein pathology in the dentate gyrus (Adamowicz *et al.,* 2017). In the present study, ring-shaped or Pick-body like NCIs were frequently found in the dentate gyrus of MSA patients with cognitive impairment. Although both Lewy body diseases and MSA are classified as synucleinopathy, the present study has clearly shown that MSA has the distinct distribution pattern of morphologically different neuronal inclusions in the hippocampus and parahippocampus compared with Lewy body diseases.

In the present study, we could not identify any correlation between NCI or GCI load in the prefrontal or temporal cortices or in their subcortical white matter. However, frontal release signs were found more commonly when patients developed FSD, indicating the involvement of frontal lobe dysfunction in the pathogenesis of FSD. Indeed, several previous postmortem studies examined the potential correlation between frontal and parietal cortical degeneration and cognitive impairment in MSA (Wakabayashi *et al*., 1998; Konagaya *et al*., 1999; Piao *et al*., 2001; Armstrong *et al*., 2007; Cykowski *et al.,* 2015). It was reported that globular NCIs in the neocortex were associated with cognitive impairment (Cykowski *et al.,* 2015). In addition, loss of neurons and myelinated fibers in deeper cortical layers of frontal and parietal lobes was evident in MSA patients with cognitive impairment and numerous GCIs were also

found in the underlying white matter (Wakabayashi *et al*., 1998; Piao *et al*., 2001). On the other hand, Armstrong et al. showed vacuolation of glial cells in the frontal cortex in patients with cognitive impairment (Armstrong *et al*., 2007). Recently, Fiorenzato et al. have performed magnetic resonance imaging volumetric, clinical and cognitive assessments of 72 patients with MSA, revealing significant atrophy in the left dorsolateral prefrontal cortex (Fiorenzato *et al*., 2017). These findings still support the association of FSD with the disruption of the frontal-subcortical circuit. Further investigation of white matter myelin loss as a marker for cortical neuronal loss may shed light on topographic correlates of FSD.

Due to the scarcity of clinicopathological studies using a large number of postmortem MSA cases, it is important to know the exact number of patients with cognitive impairment and their clinical presentations. In the Koga et al study, cognitive impairment was found in 32% of 102 pathologically proven MSA. In their report, MSA patients who had either neuropsychological evaluations or cognitive screening tests showed deficits in processing speed (75%) and executive dysfunction (69%), followed by MI (31%), visuospatial (18%) and language (12%) (Koga *et al*., 2017). On the other hand, Cykowski et al., reported MI in 60% (N = 6/10) of autopsy-proven MSA patients with cognitive impairment (Cykowski *et al.,* 2015). Despite being a rather rare clinical manifestation, some patients with MSA can also develop frontotemporal dementia (Aoki *et al*., 2015). In the present study, 30 of 148 (20.3 %) MSA patients developed cognitive impairment. Among these 30 patients, 63.3% (N = 19/30) developed executive dysfunction, 60% (N = 18/30) MI, 23.3% (N = 7/30) impaired processing speed, 20% (N = 6/30) personality change, and 6.7% (N = 2/30) disinhibition. In addition, two patients had a deficit in language function (impairment of naming ability with semantic errors (case 6) and nominal dysphasia (case 30)). It should be noted that our brain bank receives more cases through specialist clinics, which can be different from other studies. Thus, institutional bias can affect the frequency, degree and type of cognitive impairment in study cohorts.

However, the present study suggests that patients with MSA can develop MI as frequently as FSD. In addition, up to 20% of MSA patients with cognitive impairment had some clinical features suggestive of frontotemporal dementia, including personality change or disinhibition. Although these symptoms are considered to be mild in severity compared to those of other patients with a clinical diagnosis of frontotemporal dementia, the presence of these symptoms might not be as rare as thought previously.

There have been debates regarding cognitive impairment and disease duration in MSA. Chang et al. examined 23 patients with clinically probable MSA and showed that scores on clinical dementia rating and MMSE inversely related to disease duration (Chang *et al*., 2009). Furthermore, Brown et al. reported that, among patients surviving for ≥ 8 years, almost half developed some degree of cognitive impairment (Brown *et al.,* 2010). Indeed, we have experienced that rare MSA patients with unusually long disease duration have developed cognitive impairment after 13.5 and 17 years (Petrovic *et al*., 2012). However, in the present study, there was no difference in disease duration between patients with or without cognitive impairment (MSA-NC: 7.2 ± 3.0 years; MSA-FSD: 8.0 ± 3.1 years; MSA-MI: 8.8 ± 6.9 years; MSA-MI+FSD: 8.0 ± 2.5 years). In addition, of 64 patients who survived for 8 years or more, 15 (23.4%) had cognitive impairment, which is similar to the proportion of cognitive impairment in the total number of MSA patients (20.3%, N = 30/148). Again, our results showed that, regardless of disease duration, patients with MI had more NCIs than those with NC. Thus, additional factors besides disease duration might be necessary to trigger cognitive dysfunction in MSA.

In the present study, there was no significant difference in the scores or frequencies of concomitant pathologies including NFTs, amyloid plaques and Lewy bodies between patients with or without cognitive impairment. In addition, no difference in the frequencies of vascular disease likely to contribute cognitive impairment was found among the groups. Geser et al.

reported that 4 (14%) out of 29 pathologically proven MSA cases had a low level of TAR DNA-binding protein 43 (TDP-43, encoded by TARDBP) positive inclusions, located chiefly in medial temporal lobe and subcortical areas. They suggested that clinical symptoms including behavioural and cognitive features might not result from TDP-43 pathology (Geser *et al*., 2011). More recently, in a series of 148 MSA cases from the Mayo Clinic Brain Bank, only 10 (7%) were reported to have concomitant TDP-43 pathology. Considering patients harboring concomitant TDP-43 pathology were significantly older at age of death than patients without TDP-43 pathology, concomitant TDP-43 pathology in MSA was considered to be age-related (Koga *et al*., 2018). Furthermore, APOE Ɛ4 alleles, a genetic risk factor for Alzheimer's disease pathology, were less frequent in patients with MSA than control cases (Robinson *et al*., 2018). C9orf72 GGGGCC hexanucleotide repeat expansion, one of the major genetic causes of frontotemporal lobar degeneration-TDP, was not found in any MSA cases (Robinson *et al*., 2018). Thus, concomitant pathologies including NFTs, amyloid plaques, Lewy bodies, TDP-43 positive inclusions and cerebral vascular pathology are unlikely to have triggered cognitive impairment including FSD and MI.

There are several limitations to the present study. First, cognitive dysfunction in these patients with MSA could well have been underestimated because not all patients underwent formal cognitive assessments. Second, due to the nature of retrospective post-mortem studies, longitudinal and/or systemic cognitive evaluation is not available. Thus, the presence of mild cognitive impairment could have been overlooked, especially in patients who were reported to have had normal cognition. The prevalence of cognitive impairment may vary between studies based on the methodology including clinical setting, clinical vs. pathological diagnosis of MSA, and the definition of cognitive impairment. In a large clinicopathological study at the Mayo clinic by Koga et al, of the 102 pathologically proven MSA cases, 33 (32%) developed cognitive impairment (Koga *et al*., 2017). Patients were deemed to have cognitive impairment

based on the diagnostic impressions of clinicians as well as self-reported cognitive decline (Koga *et al.*, 2017). In the present study, we did not include patients' subjective complaints or care-givers' assessment as a part of the criteria for cognitive impairment. Other recent studies that investigated cognitive impairment in patients with a clinical diagnosis of MSA have shown heterogeneous frequencies of cognitive impairment ranging from 15 to 46% (Brown et al., 2010; Stankovic et al., 2014; Caso et al., 2019). Our recent study showed that only 160 out of 203 cases (79%) with the clinical diagnosis of MSA were correctly diagnosed in life and had pathologically confirmed MSA. The remaining 21% had other pathological conditions, mainly PD or progressive supranuclear palsy. These findings suggest that many studies of cognitive impairment using clinically diagnosed MSA patients may erroneously include PD or progressive supranuclear palsy cases, in which cognitive impairment is more prevalent. (Green et al., 2002; Aarsland and Kurz, 2010; Brown *et al.*, 2010; Miki *et al.*, 2019). Therefore, differences in the criteria for cognitive impairment, other sampling criteria and clinical settings between our study and others are likely to contribute to discrepancies in the prevalence of cognitive impairment. Retrospective review of the medical record is another limitation that is shared between all clinicopathological studies in the literature (Koga *et al.*, 2015; Koga *et al.*, 2017). Differing cognitive tests are used, and at varying intervals. Moreover, small sample sizes may also lead to discrepancies in prevalence of cognitive impairment. Prospective autopsy-proven studies will be required to resolve the above limitations.

In conclusion, we have demonstrated that NCI burden in the hippocampus and parahippocampus contributes to the occurrence of MI in MSA. Compared to MSA patients without cognitive impairment, patients with MI develop more NCIs in these regions regardless of disease duration.

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Competing interests

YM, HL, NQ and JLH are members of movement disorder society MSA criteria revision task force.

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Figure legends

Fig. 1

Grading scales of NCIs and GCIs for semi-quantitative analysis. The numbers of NCIs and GCIs were evaluated based on a four-point scale. Examples of 1+ (sparse) (A, D, G), 2+ (moderate) (B, E, H) and 3+ (severe) (C, F, I). The different frequencies in GCIs indicate 0 (0-5 GCIs); 1+ (6-19 GCIs) (J); 2+ (20-39 GCIs) (K); 3+ (≥40) (L). A-L: α-synuclein immunohistochemistry. Bars = 40 μm.

Fig. 2

Representative NCIs in the hippocampusand and parahippocampus, and the neocortex. Ring-shaped NCIs (A) and Pick body-like NCIs (B) in the dentate gyrus. Neurofibrillary tangle-like NCI in the CA2 region (C). Ring-shaped NCIs in the superficial layer (D) (arrows), and perinuclear (E) and globular (F) NCIs. A-F: α-synuclein immunohistochemistry. Bars = 10 μm (A-F).

Fig. 3

Semi-quantitative analysis of neuronal cytoplasmic inclusions (NCIs) in the hippocampus and parahippocampus in multiple system atrophy (MSA) patients with normal cognition (NC) (N = 118), frontal-subcortical dysfunction (FSD) (N = 12) and memory impairment (MI)/MI+FSD (pure MI: N = 6; MI+FSD: N = 12). Significant increase in NCIs was found in the dentate gyrus, cornu ammonis regions (CA) 1-4, and entorhinal cortex in patients with MI/MI+FSD compared to patients with NC (A-E, and G). There is also a greater burden of NCIs in CA4 of patients with MI/MI+FSD compared to patients with FSD (E). * $P < 0.05$; ** $P < 0.01$.

Fig. 4

Semi-quantitative analysis of NCIs in the hippocampus and parahippocampus based on pathological subtypes (MSA-striatonigral degeneration (SND), MSA-olivopontocerebellar atrophy (OPCA) and MSA-mixed). In MSA-mixed pathological group, patients with MI/MI+FSD had more NCIs in the dentate, CA1, CA2, CA4 and subiculum (A, B, C, E, and F). MSA-SND subtype (NC: N = 46; FSD: N = 2; MI: N = 0; MI+FSD: N = 3). MSA-OPCA subtype (NC: N = 33; FSD: N = 6; MI: N = 3; MI+FSD: N = 8). MSA-mixed subtype (NC: N = 39; FSD: N = 4; MI: N =3; MI+FSD: N = 1). * $P < 0.05$; ** $P < 0.01$.

Fig. 5

Semi-quantitative analysis of NCIs in the prefrontal, temporal and cingulate cortex and amygdala. The numbers of NCIs in these regions were not different between patients with NC (N = 18), FSD (N = 12) and MI/MI+FSD (pure MI: N = 6; MI+FSD: N = 12).

Fig. 6

Semi-quantitative analysis of glial cytoplasmic inclusions (GCIs) in the prefrontal and temporal white matter and cingulate bundle. There was no statistical difference in the number of GCIs between patients with NC (N = 18), FSD (N = 12) and MI/MI+FSD (pure MI: N = 6; MI+FSD: N = 12).

Supplementary figures

Supplementary Fig. 1

Neuropathological findings of case 30. (A, B) Gross examination revealing slight frontal lobe atrophy and the atrophy of the temporal lobe, which is most apparent in the anterior temporal

lobe (arrowheads) and involves the medial temporal lobe structures (arrows). α-synuclein immunohistochemistry revealing numerous ring-shaped NCIs in the dentate gyrus (C), neurofibrillary tangle-like NCIs in CA1 (D) and many NCIs in the frontal cortex (E). Bars = 100 μm (C-E).

Supplementary Fig. 2

Semi-quantitative analysis of NCIs in the hippocampus and parahippocampus in patients with NC (N = 116), FSD/MI+FSD (FSD: N = 12; MI+FSD: N =12) and MI (N = 6). Significant increase of NCIs was found in the dentate gyrus, CA2 and CA4 in patients with MI compared to patients with NC (A, C, and E). On the other hand, a significant increase of NCIs in entorhinal cortex is found in patients with FSD/MI+FSD compared to patients with NC. There is no difference in NCIs in the same region between patients with FSD/MI+FSD and MI (E). * $P < 0.05$; ** $P < 0.01$.

Supplementary Fig. 3

Semi-quantitative analysis of NCIs in the hippocampus and parahippocampus between patients with NC (N = 39), FSD/MI+FSD (FSD: N = 4; MI+FSD: N = 1) and MI (MI: N =3) in MSA-mixed pathological subgroup. There are more NCIs in the dentate gyrus, CA1, CA2 and CA4 in patients with MI than patients with NC. * $P < 0.05$