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DISTRIBUTION OF NEURONAL CYTOPLASMIC INCLUSIONS IN MULTIPLE SYSTEM ATROPHY

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ABSTRACT

Neuronal cytoplasmic inclusions (NCIs) were demonstrated by means of silver staining (Gallyas staining) in the central nervous systems of 18 deceased patients with multiple system atrophy (MSA) − 6 with olivo-pontocerebellar atrophy (OPCA), 6 with striatonigral degeneration (SND) and 6 with Shy-Drager syndrome (SDS). We observed NCIs in the cerebral cortex, putamen, pons, medulla oblongata and spinal cord, and especially in the putamen and pons of all cases with MSA. No NCIs were observed in the cerebellum and midbrain. The findings were common to all 3 subtypes of MSA. NCIs were not present in patients with other neurodegenerative disorders and non-neurological disorders. Our findings indicate that NCIs represent a special neuronal alteration characteristic of MSA and support the theory that OPCA, SND and SDS represent manifestations of a single condition i.e. MSA.

Key Words: Multiple system atrophy, Neuronal cytoplasmic inclusions, Gallyas staining

INTRODUCTION

Papp et al.[1)] have reported the presence of oligodendroglial argyrophilic inclusions, otherwise known as glial cytoplasmic inclusions (GCIs) in patients with MSA, providing the first direct evidence that OPCA, SND and SDS are variants of the same disease − MSA. Soon afterward, the presence of argyrophilic NCIs was demonstrated in the neurons of pontine nuclei taken from patients with sporadic OPCA.[2)] We examined 18 cases with MSA, other neurodegenerative diseases and non-neurological diseases in order to clarify the distribution and disease specificity of NCIs.

MATERIALS AND METHODS

Patients

The materials used for this study consisted of 18 brains and 5 spinal cords from deceased patients who were clinically diagnosed MSA (Table 1). They were classified clinico-neuropathologically into the following 3 subtypes: 6 with OPCA (cases 1−6), 6 with SDS (cases 7−12), and 6 with SND (cases 13−18). The control materials comprised of 12 brains from histologically verified cases with the following conditions: Joseph disease (1 case), dentato-rubro-pallido-luysian atrophy (1 case), Pick disease (1 case), Huntington's disease (1 case), diffuse Lewy body disease (1 case), familial amyotrophic lateral scrolosis (2 cases), amyotrophic lateral scrolosis with dementia (1 case) and non-neurological diseases (4 cases).

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Neuropathological examination

The brains and spinal cords were fixed in 10% formol saline for at least 4 weeks. The cerebrums were cut coronally, the cerebellums sagitally, the brain-stems axially at 1 cm intervals and the spinal cords segmentally. These blocks were embedded in paraffin wax, and sliced sections (8 μm thick) were stained by silver impregnation based on physical development (Gallyas method) and by the Klüber-Barrera method. Gallyas impregnation was performed as described.[3] The cerebral cortex, basal ganglia (putamen, globus pallidus), midbrain (oculomotor nucleus, red nucleus and substantia nigra), pons, cerebellum (cortex, medulla, Purkinje cell and dentate nucleus), medulla oblongata and spinal cord were examined by light microscopy.

RESULTS

Table 1 shows the clinical features (cerebellar signs, Parkinsonism and autonomic failure) and neuropathological alterations (macroscopic changes, cell loss and gliosis) in patients with MSA. Since these changes were previously reported,[4] the following description is mainly limited to findings relevant to NCIs.

Briefly, we will present the pathological changes in MSA. In 14 of the 18 brains, the pathological alterations were typical of OPCA i.e. significant atrophy of the basis pontis and cerebellum, a decrease in the number of Purkinje cells and moderate to servere cell loss in the inferior olivary nucleus et al. (Fig. 1-a, b).

In 16 of the 18 brains, we found pathological alterations typical of SND i.e. schrinkage and discoloration of the putamen (Fig. 1-c), loss of the nerve cells and gliosis in the putamen and substantia nigra (Fig. 1-d).

In all of the 14 medullas and 14 spinal cords examined, there were histopathological alterations of the autonomic nervous system i.e. a loss of nerve cells in the dorsal vagal nucleus, intermediolateral column and Onuf nucleus (Fig. 1-e, f).

NCIs in the pontine cells were round or ovoid with a clear-cut border and their nuclei were displaced to the periphery (Fig. 2-a). NCIs in other structures were varied in shape: ovoid (Fig. 2-b), reniform (Fig. 2-c), crescent-shaped (Fig. 2-d) and sometimes flamed-shaped (Fig. 2-e).

The distribution of NCIs in MSA is shown in Table 2 and the summary in Table 3.

Silver staining (Gallyas staining) revealed NCIs in all of the 18 brains from the cases with MSA irrespective of the clinical or histopathological findings.

In the cerebral cortex, a majority of the NCIs were found in the deep layer adjacent to the medulla. NCIs were also found in the putamen and pons in all of the cases with MSA. In the putamen, NCIs were present exclusively in the putaminal small nerve cells. NCIs were also found in the pontine nuclei of all cases with MSA. In some cases, the inferior olivary nucleus and arcuate nucleus of the medulla oblongata had only a small number of surviving neurons, but NCIs were observed in most of the examined inferior olivary nuclei and some of the arcuate nuclei. NCIs were present in the anterior horn cells of the spinal cord, but not in the intermediolateral column cells of the thoracic cord.

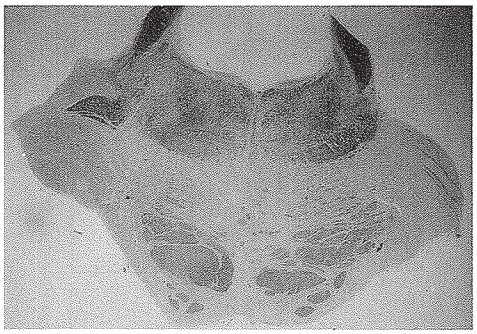
In contrast, NCIs were absent in the globus pallidus, cerebellum (cortex, medulla, Purkinje cell and dentate nucleus) and midbrain (oculomotor nucleus, red nucleus and substantia nigra).

These findings were present in all cases, and there were no definite differences between the 3 MSA subtypes. NCIs were not present in the cases with other neurodegenerative diseases and non-neurological disorders.

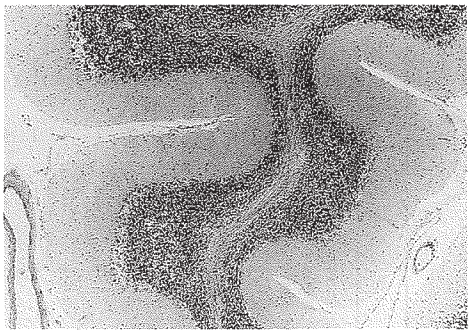
Table 1. Clinical and Pathological Details of the Patients

Clinical subtype	Case No.	Age at onset/ sex	Duration (yrs)	Cerebellar signs	Parkinsonism	Autonomic failure	Cell or fiber loss															
							Caudate nucleus	Putamen	Globus pallidus	Substantia nigra	Locus coeruleus	Pontine nucleus	Cerebellar white matter	Purkinje cells	Dentate nucleus	Inferior olivary nucleus	Vestibular nuclei	Dorsal vagal nucleus	Intermediolateral column cell	Anterior horns	Clarke's column	Pyramidal tracts
OPCA																						
	1	59/F	4.5	+	+	+	−	++	+	−	++	++	+	+	−	+	−	++	++	−	−	−
	2	57/M	6.0	+	+	+	−	+	−	+	+	++	++	+	−	+	0	0	0	0	0	0
	3	58/M	7.0	+	+	−	−	−	−	−	+	+	++	++	−	++	+	+	+	−	−	+
	4	62/M	7.0	+	+	+	+	++	+	++	++	+	+	+	−	+	+	+	++	−	−	+
	5	53/M	8.0	+	−	+	−	+	−	++	+	+	+	+	−	+	−	+	+	−	−	+
	6	43/M	10.0	+	−	+	−	++	+	++	+	++	++	++	−	++	−	+	+	++	+	++
SDS																						
	7	66/M	3.0	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	++	+	−	+
	8	50/F	4.0	+	+	+	−	++	+	+	++	+	+	+	−	+	0	0	+	−	−	−
	9	42/M	5.0	+	+	+	−	+	−	++	+	++	+	+	−	+	++	++	+	−	−	+
	10	63/M	5.0	+	+	+	−	−	−	+	+	−	−	+	−	−	+	+	++	−	−	−
	11	56/M	7.0	+	−	+	−	+	−	+	++	−	+	+	−	+	0	0	++	−	−	+
	12	50/M	8.0	+	+	+	−	++	+	++	++	+	+	+	−	++	−	+	+	−	−	+
SND																						
	13	63/F	1.5	−	+	+	−	++	+	+	+	+	+	+	−	−	+	+	0	0	0	0
	14	52/F	2.5	−	+	+	−	++	++	++	+	−	−	−	−	−	−	++	0	0	0	0
	15	57/F	4.0	−	+	+	−	++	−	+	+	+	−	+	−	−	−	+	++	−	−	+
	16	69/F	4.0	+	+	+	−	++	−	+	+	−	+	−	−	−	0	0	0	0	0	0
	17	50/F	10.0	+	+	0	−	+	−	++	+	+	++	++	−	+	−	+	+	−	−	+
	18	58/M	12.0	−	+	0	−	+	−	++	+	++	++	+	0	++	−	+	+	−	−	−

OPCA: olivopontocerebellar atrophy, SDS: Shy-Drager syndrome, SND: striatonigral degeneration, +: mild or moderate, ++: severe, −: not detected, 0: not examined.

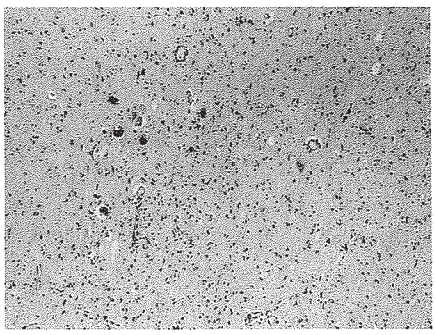


a. Significant atrophy of the basis pontis with loss of myelin in the transverse pontine fibers.

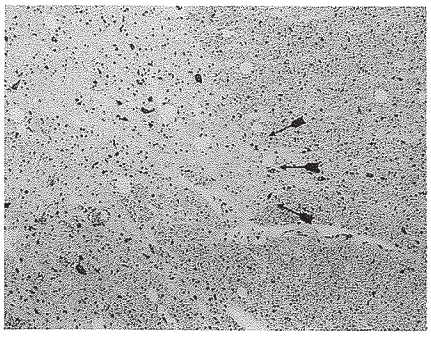


b. Significant atrophy of cerebellar cortex and medulla and decrease in the number of Purkinje cells.

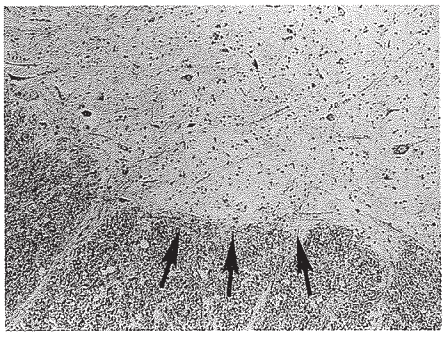
c. Definite shrinkage and pallor staining of the putamen (arrows).



d. Loss of nerve cells in the substantia nigra



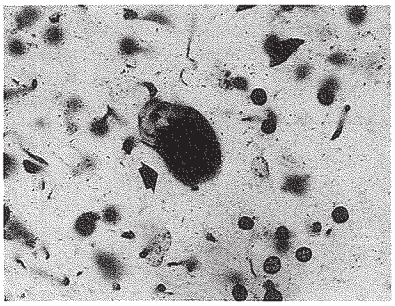
e. Severe loss of nerve cells in the intermediolateral-column (arrows).



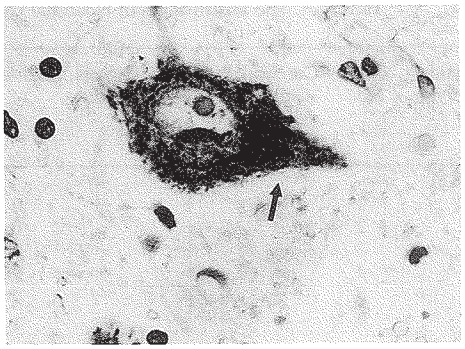
f. Severe loss of nerve cells in the Onuf nucleus (arrows).

Fig. 1.

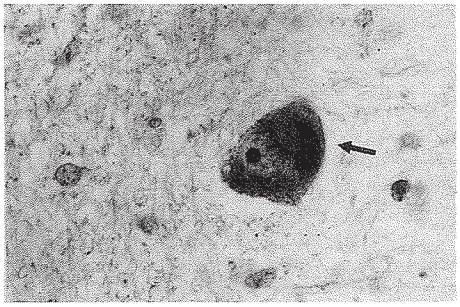
a, c: Klüber-Barrerea, b, d, e, f:hematoxylineosine



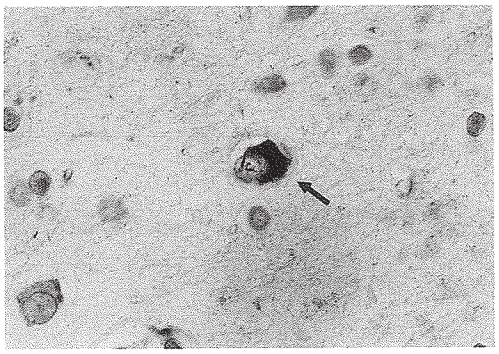
a. pontine nucleus



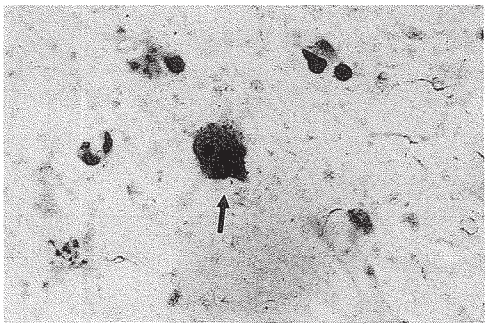
b. an anterior horn cell in the spinal cord



c. inferior olivary nucleus



d. cerebral cortex



e. a putaminal small cell

Fig. 2. Neuronal Cytoplasmic Inclusions (arrows)

Table 2. The Distribution of Neuronal Cytoplasmic Inclusions in Multiple System Atrophy

Clinical subtype	Case No.	Cerebral Cortex	Putamen	Globus pallidus	Midbrain	Pontine nucleus	Cerebellum	Inferior olivary nucleus	Arcuate nucleus	Anterior horns	Intermediolateral column
OPCA											
	1	+	+	−	0	++	0	++	+	0	0
	2	+	+	−	0	+	0	−	−	0	0
	3	++	+	−	−	++	−	++	−	0	0
	4	+	+	−	0	+	0	−	−	0	0
	5	−	++	−	0	++	0	++	+	0	0
	6	−	++	−	−	+	−	+	+	+	−
SDS											
	7	+	+	−	−	++	−	+	−	+	−
	8	−	+	−	0	+	0	+	−	0	0
	9	−	+	−	0	+	0	+	+	0	0
	10	−	+	−	0	+	0	++	+	0	0
	11	+	+	−	0	++	0	++	+	0	0
	12	+	+	−	0	+	0	+	+	0	0
SND											
	13	+	+	−	0	++	0	+	+	+	−
	14	+	+	−	−	+	−	+	+	0	0
	15	++	+	−	−	++	−	+	+	−	−
	16	+	++	−	0	++	0	++	−	0	0
	17	+	+	−	−	+	0	+	+	+	−
	18	+	+	−	0	++	−	++	−	0	0

+: mild, ++: moderate, −: not detected, 0: not examined

Table 3.

Distribution of NCI in MSA	
Cerebral cortex	13/18 CASES
Putamen	18/18 CASES
Pons	18/18 CASES
Pontine nucleus	
Medulla oblongata	16/18 CASES
Inferior olivary nucleus, Arcuate nucleus	
Spinal cord	4/5 CASES
Anterior horns	
The structures where NCI is absent	
Cerebellum	0/6 CASES
Cortex, Medulla, Purkinje cell, Dentate nucleus	
Midbrain	0/6 CASES
Oculomotor nucleus, Red nucleus, Substantia nigra	

Figure 4 schematically shows the pathologic lesions in the 3 MSA subtypes. The first subtype is OPCA, represented by case 3 and characterized by severe degeneration in the cerebellum and inferior olivary nuclei, mild or moderate degeneration in the pontine base and intermediolateral column of the thoracic cord, and slight degeneration in the substantia nigra and putamen. The second subtype is SND. Representative case 14 shows severe degeneration in the substantia nigra and putamen and slight degeneration in the cerebellum, pons and inferior olivary nuclei.

The third type is SDS, as seen in case 7. The degeneration was most severe in the intermediolateral column cells of the thoracic cord. Mild to moderate lesions were also present in the cerebellum, substantia nigra, putamen and pons.

Table 1 and Figure 4 show the primary differences in the lesions among the 3 MSA subtypes. The clinical symptomatologies were different in each subtype, especially in the early stages.

Figure 5 shows the schematic distribution of NCIs in the 3 representative cases described above. The distribution of NCIs among the 3 MSA subtypes was almost the same. In addition, our study also revealed that GCIs were present in the cerebral cortex, pons, putamen, medulla oblongata and spinal cord only in patients with MSA (Fig. 3) as previously reported.[1,2,12–14)]

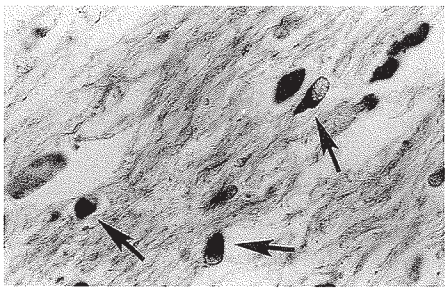


Fig. 3. Glial Cytoplasmic Inclusions in a pontine nucleus (arrows)
(Fig. 2,3; Gallyas and Klüber-Barrerea double staining)

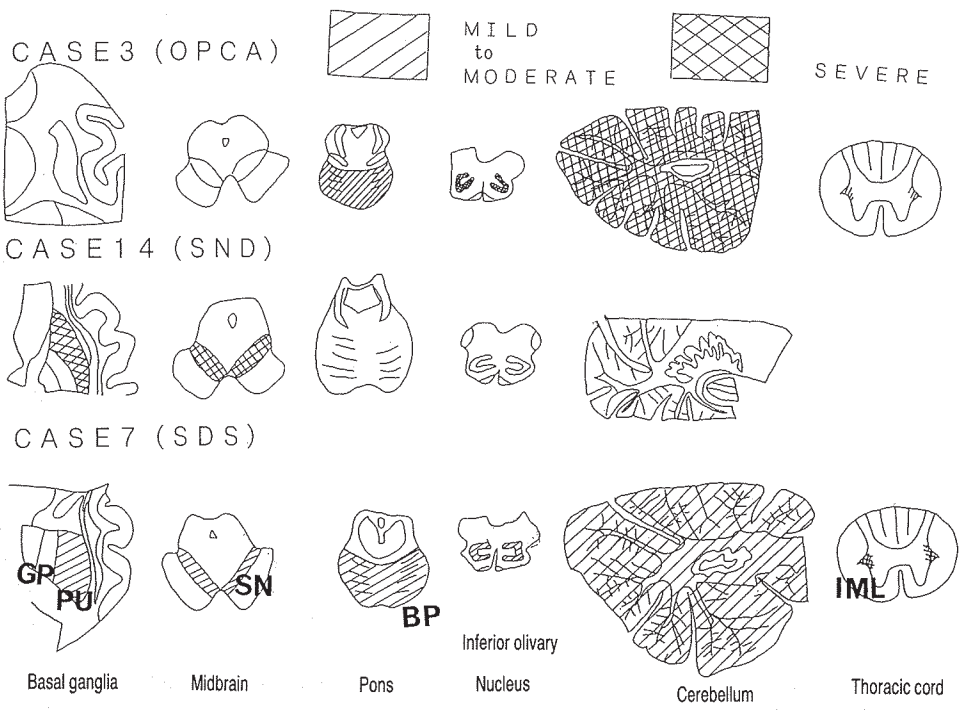


Fig. 4. Lesions in the three subtypes of MSA

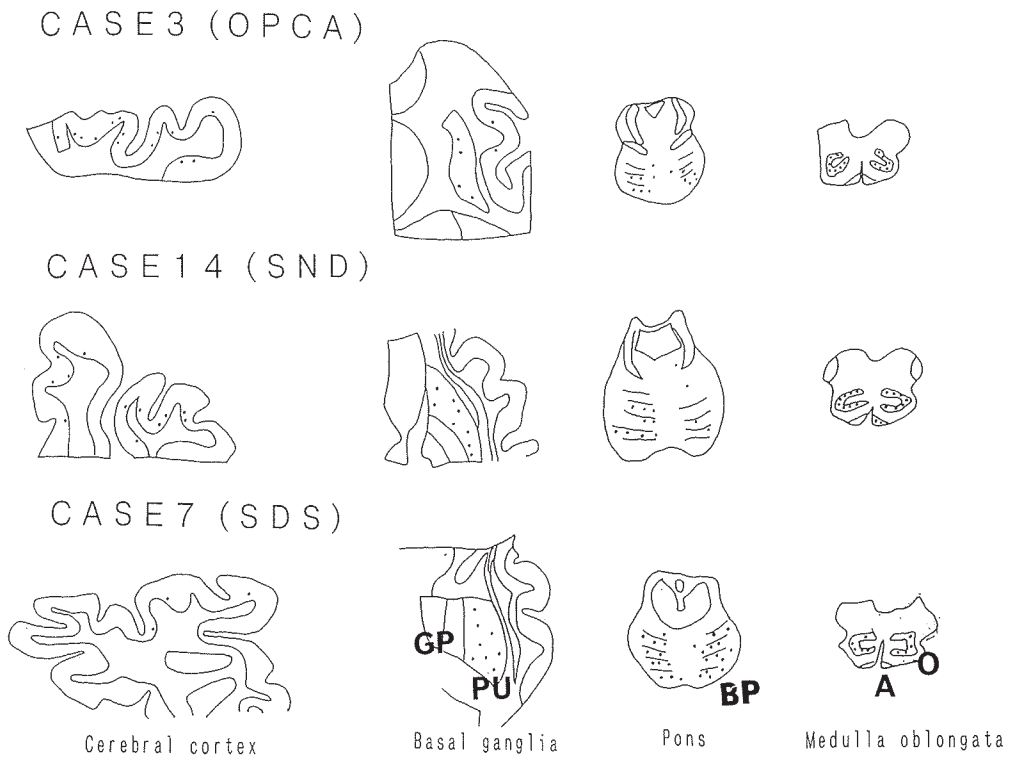


Fig. 5. The distribution of NCIs in the three subtypes of MSA

Abbreviations: PU; putamen GP; globus pallidus SN; substantia nigra BP; basis pontis O; Inferior olivary nucleus A; arcuate nucleus IML; intermediolateral column (Fig. 4,5).

DISCUSSION

In 1900, Dejerine and Thomas[5] coined a descriptive name "olivopontocerebellar atrophy" which begins in middle age and has cardinal pathological lesions in the cerebellar cortex, inferior olivary nuclei and pontine nuclei. Most cases encountered were sporadic.

The term "striatonigral degeneration" was first proposed by Adams and co-workers[6,7] to describe 4 patients with Parkinsonian syndrome in which postmortem examination disclosed marked degeneration of the substantia nigra in addition to those of the striatum, olives and cerebellum.

In 1960, Shy and Drager[8] described a distinct neurological syndrome manifested by features of autonomic failure such as orthostatic hypotension, urinary and rectal incontinence, loss of sweating and impotence, in combination with parkinsonism and cerebellar signs. Post-mortem examination showed marked neuropathological changes in the caudate nucleus, substantia nigra, olives, cerebellum and intermediolateral columns of the spinal cord; the putamen was also affected.

In 1969, Takahashi et al.[9] presented the first report on patients with SDS in Japan. They stated: "reviewing the literature and analysing the present cases, it becomes evident that considerable common clinical and pathological features exist in both OPCA and SDS. It is strongly

suggested that both OPCA and SDS belong to a category of presenile system-degenerative diseases of the central nervous system of unknown etiology and that they are nosologically allied conditions."

In 1969, Graham and Oppenheimer[10] proposed the use of the term "multiple system atrophy" to emphasize the clinical and pathological overlap between patients presenting with cerebellar ataxia (OPCA), parkinsonian syndrome (SND) and autonomic symptoms (progressive autonomic failure). The advantage of this term is that initially, pure clinical examples of each of the 3 MSA subtypes may go on to develop symptoms seen with the other two subtypes and even if they do not, they often have evidence at postmortem examination of multisystem involvement.

In 1989, this view received strong support from the description by Papp et al[1] of a specific cytological hallmark of MSA, oligodendroglial inclusions or GCIs. These inclusions are present in MSA, but absent from cases of familial OPCA, Parkinson's disease and other 'multisystem degenerations' such as progressive supranuclear palsy.

In 1990, Kato and Nakamura[11] reported cytoplasmic argyrophilic inclusions in pontine neurons. Further studies of NCIs have been published by a number of groups[11–13] but these vary as to details.

Kato reported cytoplasmic argyrophilic inclusions in the neurons of pontine nuclei in 6 of 14 OPCA patients. Iwabuchi found NCIs in pontine neurons in only 2 of 12 MSA cases, while Mochizuki[14] reported no argentophilic inclusions in the neurons of patients with MSA.

In our study, NCIs were detected in all of the MSA cases. In particular, NCIs were found in the putamen and pontine nuclei in every case of MSA. As to the topography of NCIs, there was no difference between the 3 MSA subtypes. Our data strongly suggests that OPCA, SND and SDS are different phenotypes of the same disease entity i.e. MSA. Our study also disclosed that NCIs were absent in other neurodegenerative diseases and non-neurological disorders. These results support the idea that the appearance of NCIs, as well as GCIs, is one of the specific diagnostic hallmarks of MSA.

Kato and Ohama[15] conducted ultrastructural, immunohistochemical and immunoelectron microscopic studies on neuronal and oligodendroglial inclusions in cases with OPCA and reported immunohistochemically neuronal and oligodendroglial inclusions are different in spite of their ultrastructural similarities. Ultrastructurally, neuronal inclusions consisted of 24 to 40 nm granule-coated fibrils, occasionally intermixed with neurofilaments. Oligodendroglial inclusions were also composed of granule-coated fibrils, 24–40 nm in diameter, similar to those of neuronal inclusions. Immunohistochemical studies revealed that the α B-crystallin epitope is expressed in almost all oligodendroglial inclusions, but not in neuronal ones. Both inclusions reacted with antibodies to ubiquitin, but not with antibodies against stress-response protein 27 (srp27) or srp 72.

Determination of the structure of NCIs and GCIs might lead to identification of the etiology of MSA. Despite the specificity of NCIs for cases of MSA in this study, the regional distribution of NCIs differed from the sites regarded as being most frequently damaged in MSA. This difference in distribution raises questions as to the role of NCIs in the pathogenesis of MSA.

These issues indicate the need for further fine-structural, immunohistochemical and biochemical investigations of NCIs, GCIs and the cells containing these inclusions.

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