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The spectrum of pathological involvement of the striatonigral and olivopontocerebellar systems in multiple system atrophy: clinicopathological correlations

Tetsutaro Ozawa,[1] Dominic Paviour,[3,5] Niall P. Quinn,[4] Keith A. Josephs,[1,5] Hardev Sangha,[1] Linda Kilford,[1] Daniel G. Healy,[2] Nick W. Wood,[2] Andrew J. Lees,[3,6] Janice L. Holton[1] and Tamas Revesz[1]

[1]*Queen Square Brain Bank,* [2]*Neurogenetics,* [3]*The Sara Koe PSP Research Centre, Department of Molecular Neuroscience,* [4]*Sobell Department of Motor Neuroscience and Movement Disorders,* [5]*Dementia Research Centre, Institute of Neurology, UCL and* [6]*The Reta Lila Weston Institute of Neurological Sciences, Windeyer Building, Cleveland Street, London, UK*

Correspondence to: Professor Tamas Revesz, Queen Square Brain Bank, Department of Molecular Neuroscience, Institute of Neurology, Queen Square, London WC1N 3BG, UK
E-mail: t.revesz@ion.ucl.ac.uk

Summary

Multiple system atrophy (MSA) has varying clinical (MSA-P versus MSA-C) and pathological [striatonigral degeneration (SND) versus olivopontocerebellar atrophy (OPCA)] phenotypes. To investigate the spectrum of clinicopathological correlations, we performed a semi-quantitative pathological analysis of 100 MSA cases with well-characterized clinical phenotypes. In 24 areas, chosen from both the striatonigral (StrN) and olivopontocerebellar (OPC) regions, the severity of neuronal cell loss and gliosis as well as the frequency of glial (oligodendroglial) cytoplasmic inclusions (GCIs) and neuronal cytoplasmic inclusions (NCIs) were determined. Clinical information was abstracted from the patients' medical records, and the severity of bradykinesia in the first year of disease onset and in the final stages of disease was graded retrospectively. The degree of levodopa responsiveness and the presence or absence of cerebellar ataxia and autonomic symptoms were also recorded. We report that 34% of the cases were SND- and 17% were OPCA-predominant, while the remainder (49%) had equivalent SND and OPCA pathology. We found a significant correlation between the frequency of GCIs and the severity of neuronal cell loss, and between these pathological changes and disease duration. Our data also suggest that GCIs may have more influence on the OPC than on the StrN pathology. Moreover, we raise the possibility that a rapid process of neuronal cell loss, which is independent of the accumulation of GCIs, occurs in the StrN region in MSA. There was no difference in the frequency of NCIs in the putamen, pontine nucleus and inferior olivary nucleus between the SND and OPCA subtypes of MSA, confirming that this pathological abnormality is not associated with a particular subtype of the disease. In the current large post-mortem series, 10% of the cases had associated Lewy body pathology, suggesting that this is not a primary process in MSA. As might be expected, there was a significant difference in the severity of bradykinesia and the presence of cerebellar signs between the pathological phenotypes: the SND phenotype demonstrates the most severe bradykinesia and the OPCA phenotype the more frequent occurrence of cerebellar signs, confirming that the clinical phenotype is dependent on the distribution of pathology within the basal ganglia and cerebellum. Putaminal involvement correlated with a poor levodopa response in MSA. Our finding that relatively mild involvement of the substantia nigra is associated clinically with manifest parkinsonism, while more advanced cerebellar pathology is required for ataxia, may explain why the parkinsonian presentation is predominant over ataxia in MSA.

Keywords: multiple system atrophy; striatonigral degeneration; olivopontocerebellar atrophy; glial cytoplasmic inclusion; clinicopathological correlations

Abbreviations: GCI = glial cytoplasmic inclusion; GFAP = glial fibrillary acidic protein; H&E = haematoxylin and eosin; IPD = idiopathic Parkinson's disease; LFB/CV = luxol fast blue/cresyl violet; MSA = multiple system atrophy; MSA-C = multiple system atrophy cerebellar type; MSA-P = multiple system atrophy parkinsonism; NCI = neuronal cytoplasmic inclusion; NCLPS = neuronal cell loss predominance score; OPC = olivopontocerebellar; OPCA = olivopontocerebellar atrophy; QSBB = Queen Square Brain Bank; SbN = substantia nigra; SND = striatonigral degeneration; StrN = striatonigral.

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Introduction

Multiple system atrophy (MSA) is a sporadic adult-onset neurodegenerative disease, which usually presents clinically as a combination of parkinsonism, cerebellar ataxia and autonomic failure. The predominant pathological correlates, olivopontocerebellar atrophy (OPCA) (Dejerine and Thomas, 1900) and striatonigral degeneration (SND) (Adams *et al*., 1961), of two forms of clinical presentation, now called MSA cerebellar type (MSA-C) and MSA parkinsonism (MSA-P), were described independently in 1900 and 1961. In OPCA, characterized clinically by predominant cerebellar ataxia, the pathological findings are primarily those of neuronal cell loss and gliosis in the inferior olivary nucleus, pontine nuclei, cerebellar hemispheres and vermis (Dejerine and Thomas, 1900), whereas SND, which has a mainly parkinsonian clinical phenotype, is associated with neuronal cell loss and gliosis in the substantia nigra (SbN), putamen, caudate nucleus and globus pallidus (Adams *et al*., 1961). Most cases of OPCA and SND are also accompanied by neurodegeneration in the autonomic nervous system which, when presenting clinically with prominent autonomic failure, has been referred to as the Shy–Drager syndrome (Shy and Drager, 1960).

Graham and Oppenheimer first proposed the term MSA based on the finding that sporadic OPCA, SND and Shy–Drager syndrome can co-exist both clinically and pathologically (Graham and Oppenheimer, 1969; Bannister and Oppenheimer, 1972; Borit *et al*., 1975; Gosset *et al*., 1983; Spokes *et al*., 1979). However, it was not until the subsequent discovery of the oligodendroglial cytoplasmic inclusions (GCIs) (Papp *et al*., 1989; Nakazato *et al*., 1990), originally identified by Gallyas silver impregnation, that the notion that MSA is a single clinicopathological entity could be confirmed. Despite the increase in our understanding of the pathological basis of MSA there are a number of important questions that remain unanswered. (i) What is the relationship between neuronal cell loss and GCIs? Papp and Lantos observed no direct correlation between the density of GCIs and the severity of neuronal loss in most anatomical sites, and they suggested that GCIs generally appear before neuronal cell loss (Papp and Lantos, 1994). Subsequently, Inoue *et al*. (1997) noted that GCIs existed abundantly in the cerebellar white matter in cases in which the rest of the olivopontocerebellar (OPC) system was well preserved, while GCIs were less frequent in cases of OPCA with severe neuronal cell loss. Although these observations raise the possibility that GCIs may play a role in the early pathogenesis of MSA, both studies were relatively small in size, therefore we re-addressed this issue. (ii) What is the spectrum of the pathological phenotypes and, in particular, what is the relative prevalence of striatonigral (StrN)- and OPC-predominant pathology in MSA? We also wished to establish whether pure SND and pure OPCA exist and whether the presumption that MSA-P has a predominantly StrN pathology and MSA-C a predominantly OPC pathology is correct. (iii) At a molecular level, both GCIs and neuronal cytoplasmic inclusions (NCIs) contain α-synuclein (Arima *et al*., 1998; Mezey *et al*., 1998; Wakabayashi *et al*., 1998*a*,*b*; Yokoyama *et al*., 2001; Sakurai *et al*., 2002), and modifications of this protein are a characteristic pathological feature of MSA (Tu *et al*., 1998; Dickson *et al*., 1999). α-Synuclein is also a major component of the Lewy body, which is the pathological hallmark lesion in idiopathic Parkinson's disease (IPD) (Spillantini *et al*., 1997). Recently the term 'synucleinopathy' has been proposed to encompass a putative common pathogenic process shared by both neurodegenerative disorders (Galvin *et al*., 2001). However, only a few reports have described the co-existence of GCIs and Lewy bodies (Hughes *et al*., 1991; Tison *et al*., 1995; Wenning *et al*., 1995; Mochizuki *et al*., 2002), therefore, the prevalence of Lewy body pathology in MSA was also determined in this study. (iv) Using our large cohort of MSA cases, we also wished to establish whether any correlation exists between clinical features and the distribution of pathological changes. In particular, we wished to investigate further the relationship between levodopa responsiveness and putaminal pathology, as previous studies have raised the possibility that the latter may be relevant (Fearnley and Lees, 1990; Wenning *et al*., 1995). In addition, a correlation between the distribution of pathology and the predominance of parkinsonism over cerebellar clinical signs was sought.

We have performed a semi-quantitative analysis of the StrN and OPC pathology in 100 pathologically confirmed MSA cases in which the clinical phenotypes were well defined in life. Furthermore, we investigated the densities of GCIs and NCIs, as well as the frequency of Lewy bodies, using α-synuclein immunohistochemistry. Finally, we evaluated the pathological spectrum of MSA and correlated this pathology with clinical findings.

Material and methods

MSA brain pathology

We systematically examined pathological material from the brains of 100 cases of MSA (46 men and 54 women), donated to the Queen

Square Brain Bank (QSBB), Institute of Neurology, London, between 1984 and 2002. Some of the brain material used in this study has also been presented in previous reports from the QSBB (previously known as the Parkinson's Disease Society Brain Research Centre; Fearnley and Lees, 1990; Hughes *et al*., 1991, 1992; Wenning *et al*., 1994*a*,*b*, 1995, 2000). The research presented in this study has been approved by the Joint Ethics Committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology.

Diagnostic procedures

Consent for post-mortem examination and brain donation was obtained from the next of kin. After removal, the brain was divided in the midline and one half was frozen and stored at −80°C. The remaining half brain was fixed in 10% formalin and sliced in the coronal plane. Tissue blocks from the frontal, temporal, parietal and occipital neocortices, in addition to basal ganglia, thalamus, amygdala, hippocampus, midbrain, pons, medulla and cerebellum, were processed in paraffin wax using standard protocols. Sections were cut at 7 μm unless otherwise stated and stained with routine methods including haematoxylin and eosin (H&E), Luxol fast blue/cresyl violet (LFB/CV) and silver impregnation (modified Bielschowsky's method). Established MSA neuropathological criteria were applied (Lowe *et al*., 1997): the presence of GCIs with neuronal cell loss and gliosis in a number of structures including the putamen, SbN, locus coeruleus, basis pontis, inferior olivary nucleus, dorsal motor nucleus of the vagus, intermediolateral column of the spinal cord (if spinal cord was available). The GCIs were routinely examined using silver impregnation.

Immunohistochemistry

Tissue sections from all areas listed above were used for immunohistochemistry using antibodies to glial fibrillary acidic protein (GFAP) and α-synuclein. Endogenous peroxidase activity was neutralized in methanol/H_2O_2 solution. Tissue sections were pre-treated with 0.1% trypsin for GFAP and with formic acid for α-synuclein immunohistochemistry. Non-specific antigen binding was blocked by normal horse serum (Vectastain elite ABC kit; Vectastain, Peterborough, UK). The primary antibodies, diluted in 1% phosphate-buffered saline (PBS) (GFAP 1 : 1000, DAKO, Ely, UK; α-synuclein 1 : 1000, Autogen Bioclear, Calne, UK), were applied and incubated for 1 h at room temperature, followed by washes in PBS solution and incubation in pre-diluted biotinylated secondary antibody. After further washes, the avidin–biotin complex (ABC) reagent (Vectastain elite ABC kit) was applied and visualization of the immunoreaction was achieved by using diaminobenzidine/H_2O_2 solution. Counterstaining was carried out with Mayer's haematoxylin.

Morphometry

Without prior information about the clinical presentation, morphometric assessment of the pathological changes in the StrN and OPC structures using a semi-quantitative approach was applied. For this, brain sections were prepared from two levels of the following anatomical areas of interest: (i) basal ganglia—the accumbens and the globus pallidus levels; (ii) midbrain—decussation of cerebellar peduncle and the red nucleus levels; (iii) cerebellum—cerebellar hemisphere and vermis; (iv) caudal and rostral pons; and (v) medulla oblongata. From these regions, 24 different brain areas of interest were chosen (Fig. 1), in which the semi-quantitative analysis was carried out by one of the authors (T.O.). Random cases were also reviewed by three further investigators (T.R., J.L.H. and K.A.J.) to ensure consistency of evaluation.

StrN pathology

Neuronal cell loss was assessed using H&E- and LFB/CV-stained sections. This permits recognition of neurons, using standard morphological criteria. Gliosis was evaluated using GFAP immunohistochemistry. A semi-quantitative assessment of neuronal cell loss and gliosis within the putamen, globus pallidus and caudate nucleus (Fig. 1) was performed, using an approach similar to that described by Wenning *et al*. (2002), and one of the following four grades was assigned to each anatomical region: absent (0), slight (1+), moderate (2+) and severe (3+). Loss of pigmented cells in six subregions of the SbN (Fig. 1) was rated as follows: absent, no free pigment (0); numbers of neurons not definitely decreased, but a small amount of free pigment is present (1+); clear neuronal loss and presence of free pigment (2+); and very few surviving neurons seen (3+). The densities of GCIs and NCIs were evaluated using a ×20 objective, and the count was taken as the average number of GCIs or NCIs in five representative fields. The semi-quantitative ratings between the number of inclusion bodies and grading scale were as follows: 0 = no inclusions, 1+ = 0–5 inclusions, 2+ = 6–19 inclusions and 3+ = ⩾20 inclusions. Examples of 1+, 2+ and 3+ severity of neuronal cell loss and densities of GCIs in the putamen are illustrated in Fig. 2.

OPC pathology

Neuronal cell loss, gliosis and densities of both GCIs and NCIs were assessed in the inferior olivary nucleus, basis pontis, cerebellar hemisphere and vermis in the manner described above (Fig. 1). In the basis pontis, using LFB/CV staining, atrophy of the transverse myelinated fibres was also assessed as follows: absent (0), slight (1+), moderate (2+) and severe (3+). In the cerebellum, the presence of torpedoes or empty baskets was graded as 1+ or 2+ neuronal cell loss, while the presence of long stretches of Purkinje cell loss was graded as 3+ neuronal cell loss.

Grading systems

The data of the semi-quantitative analysis were finally used to establish grades as follows.

StrN pathology

In our study, a published method for grading was used (Wenning *et al*., 2002). Grade 1: the SbN demonstrates 1+ or 2+ neuronal cell loss, and there is 0 or 1+ neuronal cell loss in globus pallidus, caudate nucleus or putamen. Grade 2: the SbN and putamen demonstrate 2+ or 3+ neuronal cell loss, and there is 1+ or 2+ neuronal cell loss in the caudate nucleus and globus pallidus. Grade 3: the SbN and dorsolateral putamen demonstrate 3+ neuronal cell loss.

OPC pathology

The grading scale used to characterize OPC pathology was as follows. Grade 1: in the inferior olivary nucleus, pontine nuclei, cerebellar hemisphere or vermis, there is 0 or 1+ neuronal cell loss or one structure demonstrating 2+ neuronal cell loss, while

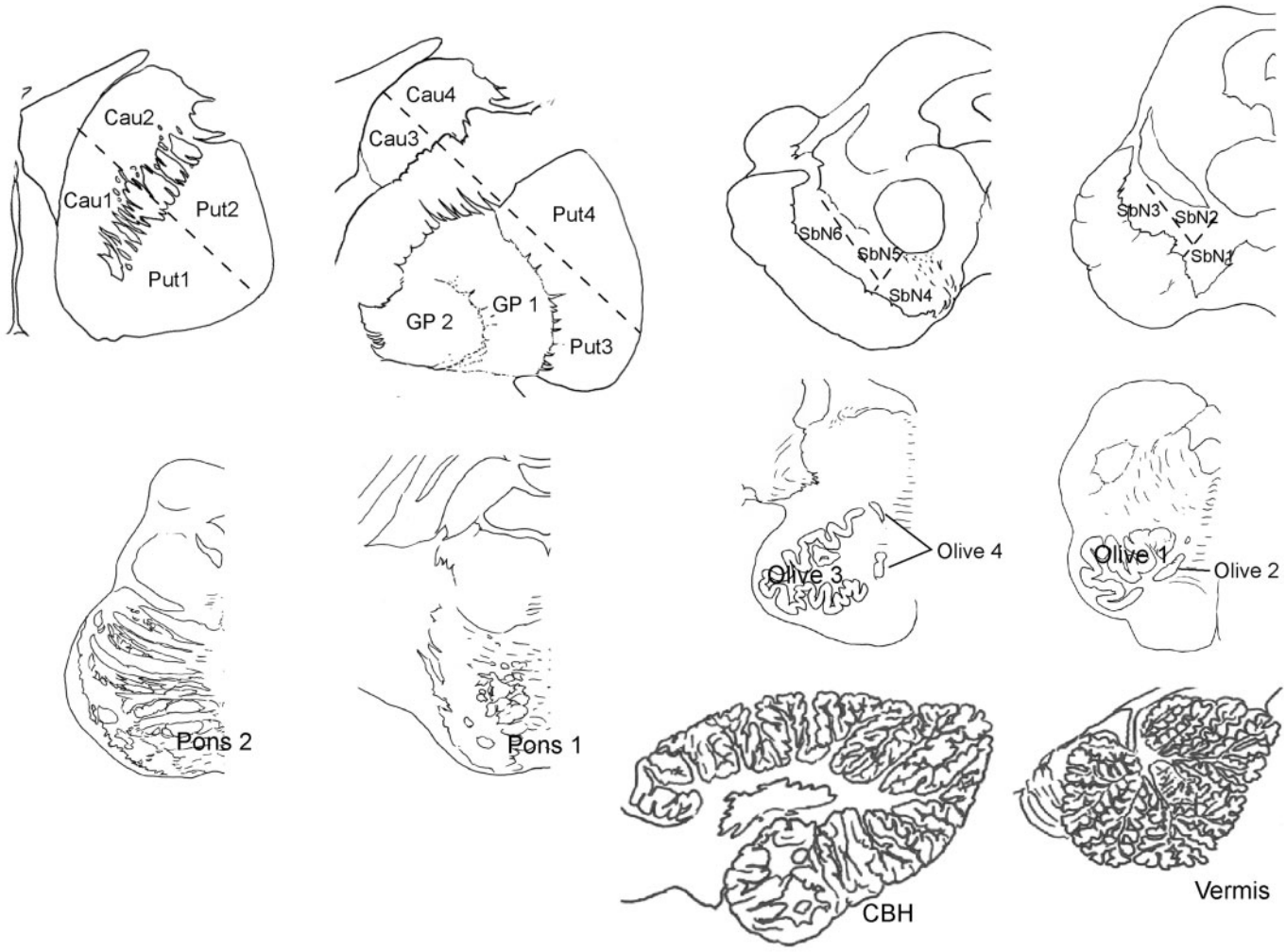

Fig. 1 Illustration of the 24 anatomical sites, in which neuronal cell loss, gliosis and the frequency of glial cytoplasmic inclusions were investigated by a semi-quantitative analysis. Cau = caudate nucleus; CBH = cerebellar hemisphere; GP = globus pallidus, Put = putamen; SbN = substantia nigra.

the others have less than 1+ neuronal cell loss. Torpedoes or empty baskets are seen in the Purkinje cell layer or granular cell layer in the cerebellum. Grade 2: in the inferior olivary nucleus, pontine nuclei, cerebellar hemisphere or vermis, there is either 2+ neuronal cell loss or one structure demonstrating 3+ neuronal cell loss, while the others have less than 2+ neuronal cell loss. Many torpedoes and empty baskets are seen in the Purkinje cell layer or granular cell layer in the cerebellum. Grade 3: more than two structures among the inferior olivary nucleus, pontine nuclei, cerebellar hemisphere or vermis demonstrate 3+ neuronal cell loss, there are very few surviving Purkinje cells in cerebellar cortex, or neurons in the inferior olivary nucleus, the transverse myelinated fibres are shrunken in basis pontis and the pontine nuclei show severe diffuse neuronal depletion.

If there were different degrees of pathology in the same structure (e.g. rostral pons 1+, caudal pons 3+), the worst (e.g. 3+) was used for the grading.

Lewy body pathology

As recent evidence in IPD suggests that Lewy bodies may first appear in the dorsal motor nucleus of the vagus, and only subsequently involve the SbN (Braak *et al*., 2003), using H&E staining and α-synuclein immunohistochemistry, we reviewed both structures and established the presence of co-existent Lewy body pathology among the MSA cases studied.

Clinical data collection

A single investigator who was blinded to the pathological data abstracted clinical information from the patients' medical records in the QSBB. Cases without complete clinical records were excluded.

The severity of bradykinesia in the first year of disease onset and in the final stages of disease was graded retrospectively as none (0), mild (1+), moderate (2+) or severe (3+) according to the investigating clinicians' comments in the records. Tremor was recorded as either present or absent at disease onset and in the final assessment. Any asymmetry of bradykinesia or tremor was noted. The presence or absence of dystonia and particularly antecollis (Rivest *et al*., 1990) was recorded, as was the presence or absence of myoclonus (Wenning *et al*., 1994*a*), perceived cognitive impairment, cerebellar ataxia and autonomic symptoms such as postural hypotension.

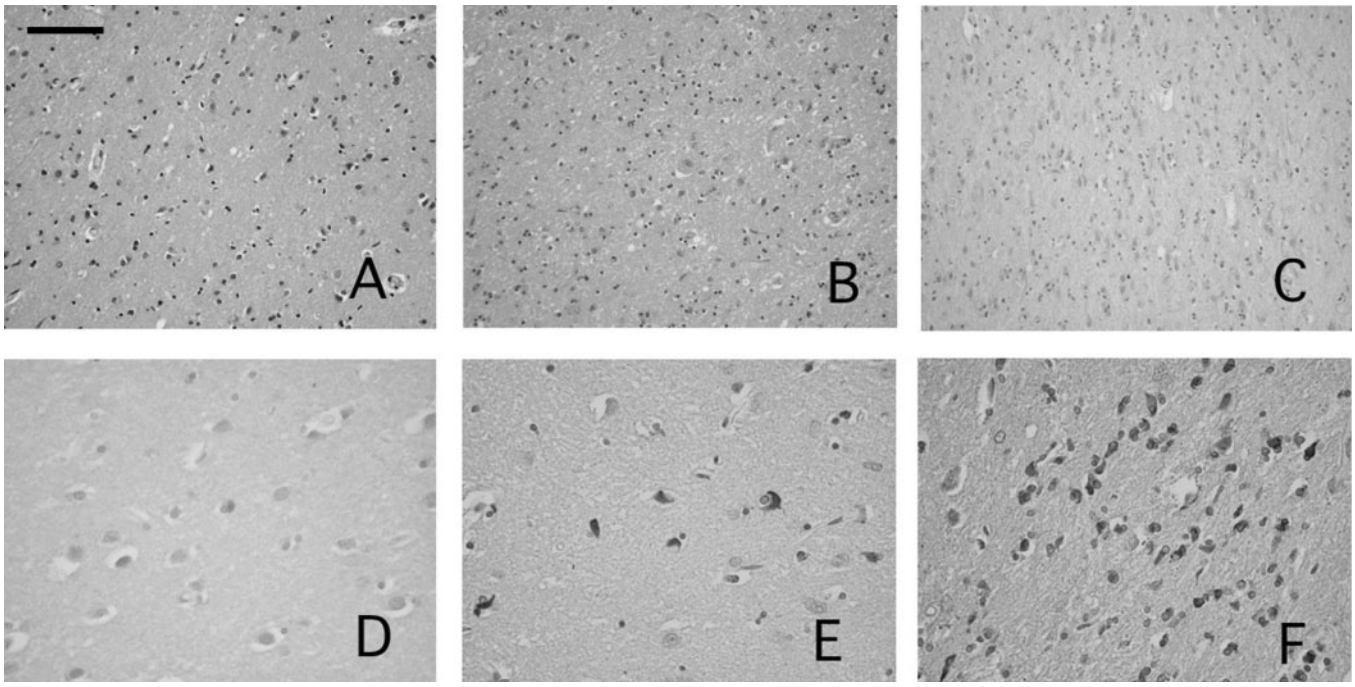

Fig. 2 The degree of neuronal loss and frequency of glial cytoplasmic inclusions were determined using a semi-quantitative approach. Examples of 1+ (slight) (**A**), 2+ (moderate) (**B**) and 3+ (severe) (**C**) neuronal cell loss in the putamen. Different frequencies of GCIs: +1 (0–5 inclusions) (**D**), +2 (6–19) (**E**) and +3 (⩾20) (**F**) are also illustrated. **A**, **B** and **C**, H&E staining; **D**, **E** and **F**, α-synuclein immunohistochemistry. The scale bar represents 40 μm in **A–C** and 20 μm in **D–F**.

Levodopa responsiveness was graded retrospectively according to a 4-point scale where (1+) equals no or a minimal response, (2+) a moderate response, (3+) a good response and (4+) an excellent response to levodopa. It has been proposed previously that the severity of pathology in the corpus striatum and particularly the putamen correlates inversely with levodopa responsiveness (Fearnley and Lees, 1990). In order to test this hypothesis, we compared the sum of the putaminal pathology scores in regions Put 1 and Put 2 in subjects grouped according to levodopa responsiveness.

Statistical analysis

Contingency tables were analysed with χ^2 test. The Mann–Whitney U test was used to compare non-parametric continuous variables for different subgroups, and relating to the clinical features seen in the different pathological subtypes of MSA. Analysis of variance (ANOVA) with Scheffe's *post hoc* test was used to compare the neuronal cell loss predominance score (see above) between the different anatomical sites. The relationships between the severity of neuronal cell loss, the grading score of GCIs and duration of the disease were analysed using Pearson's correlation coefficient. Calculations were performed using the statistical software package StatView (Abacus Concepts, Berkeley, CA).

Results

Age and gender distribution

The 100 MSA cases comprised 46 men and 54 women. The mean age at disease onset was 57.5 ± 10.5 (range 34–83) years, while the mean age at death was 64.1 ± 10.0 (range 39–87) years. The mean duration of the disease was 7.0 ± 2.9 (range 2–16) years.

General observations on the semi-quantitative analysis

The mean severity of grade for the 100 MSA cases was determined in 24 different brain regions and the data are shown in Fig. 3. Neuronal cell loss was most prominent in the dorsolateral part of the putamen, cerebellar vermis, and all sites of the SbN, with a mean value >2.5 for all these areas, whereas neuronal cell loss in the caudate nucleus was less severe (mean grade = 1.5) (Fig. 3A). Grading of the severity of GCIs showed a trend similar to that of neuronal cell loss; however, the frequency of GCIs in the SbN was not as severe as in the other structures affected by significant neuronal cell loss (Fig. 3B). Gliosis was generally severe, although moderate in the caudate nucleus, in which neuronal cell loss was mild and the number of GCIs was relatively low (Fig. 3C).

Neuronal cell loss predominance score (NCLPS)

In order to know to what extent the neuronal cell loss predominated over GCIs in different anatomical sites in MSA, mean GCI score and mean neuronal cell loss scores were calculated in the putamen, caudate nucleus, globus pallidus,

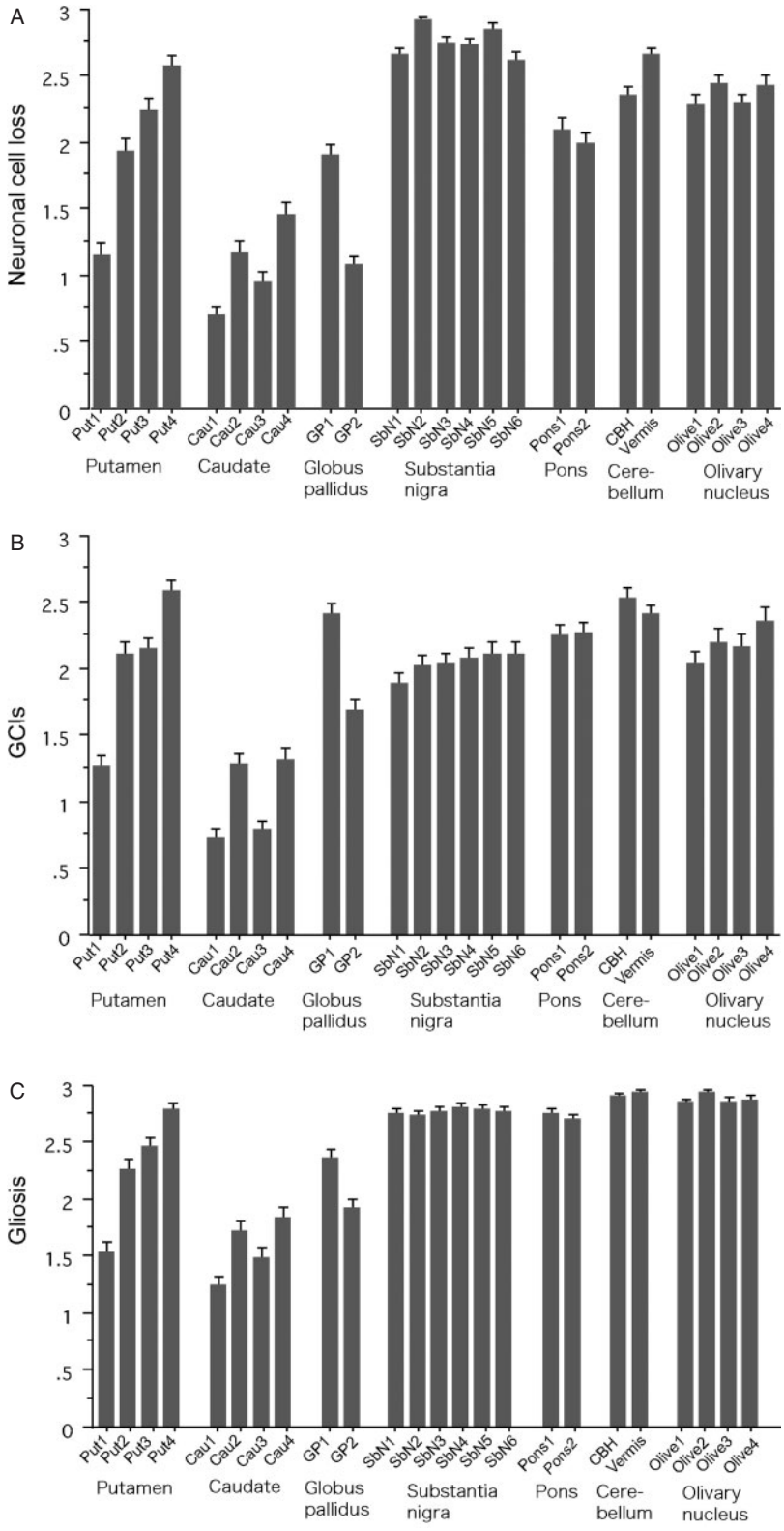

Fig. 3 Grading scores of neuronal cell loss (**A**), GCIs (**B**) and gliosis (**C**) in 24 sites of the brain structure. Data are represented as mean ± SE.

SbN, pontine nucleus, cerebellum and olivary nucleus in each case, and the mean GCI score was subtracted from the mean neuronal cell loss score. The resultant score was designated as the neuronal cell loss predominance score (NCLPS). The NCLPS in each anatomical site was compared using ANOVA with Scheffe's *post hoc* test. We found that the NCLPS of the SbN was significantly higher than that of any other anatomical sites in MSA ($P < 0.005$) (Fig. 4).

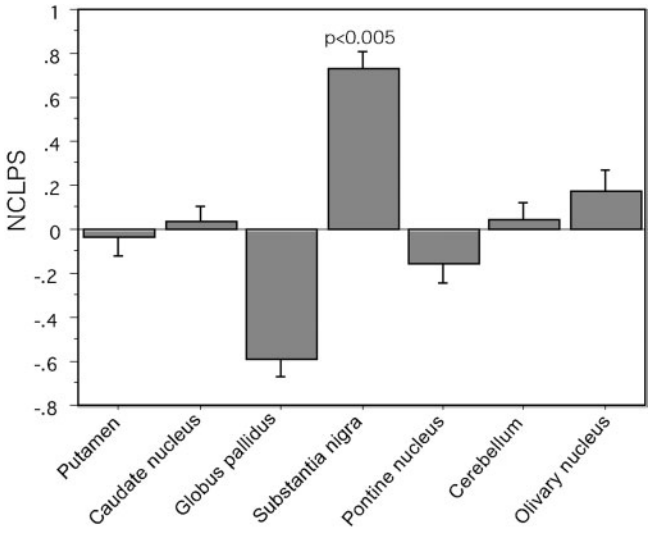

Fig. 4 The 'neuronal cell loss predominance score' (NCLPS) in different anatomical sites in MSA. The NCLPS is obtained by subtracting the mean GCI score from the mean neuronal cell loss score in each anatomical site. ANOVA with Scheffe's *post hoc* test was used to compare the NCLPS in different anatomical sites and it was found that this was significantly higher in the substantia nigra than in any other anatomical sites ($P < 0.005$). Data are represented as mean ± SE.

Correlation between GCIs, neuronal cell loss and disease duration

We investigated whether there was a relationship between neuronal cell loss and the GCI severity grading, as well as between either of these two morphological features and disease duration, in both the StrN and OPC regions. These analyses demonstrated a significant correlation between the grading score of GCIs and that of neuronal cell loss in both the StrN ($r = 0.50$, $P < 0.0001$) (Fig. 5A) and OPC regions ($r = 0.51$, $P < 0.0001$) (Fig. 5B). Furthermore, there was a correlation between the grading score of neuronal cell loss and duration of the disease in the OPC region ($r = 0.22$, $P < 0.02$) (Fig. 5C), but only a trend in the StrN region ($r = 0.18$, $P = 0.07$) (Fig. 5D). A significant correlation was found between the grading score of GCIs and disease duration in both the StrN ($r = 0.30$, $P = 0.002$) (Fig. 5E) and OPC regions ($r = 0.23$, $P = 0.02$) (Fig. 5F).

Comparison of GCIs score between StrN and OPC system

We also wished to determine any potential difference in the average GCI scores that may exist between the StrN and OPC regions in this series of MSA cases. Statistical analysis (Mann–Whitney U test) showed that the GCI grading score in the OPC region was significantly higher than that in the StrN region ($P < 0.0001$) (Fig. 6).

Pathological phenotypes of MSA

Based on the grading system described above, none of the MSA cases received a score of 0 in either the StrN or OPC region, indicating that neither pure SND nor pure OPCA exists, at least when end-stage cases are examined pathologically. In total, seven combinations of grade were identified (Table 1). For the purposes of this study, SND3–OPCA2, SND3–OPCA1 and SND2–OPCA1 were classified as pathologically StrN predominant, and re-designated as 'SND-type' MSA, while the SND1–OPCA3 and SND2–OPCA3 cases, in which OPC pathology was predominant, were designated as 'OPCA-type' MSA. The SND3–OPCA3 and SND2–OPCA2 cases, in which StrN and OPC pathology were equally severe, were re-designated as 'SND = OPCA type'. The relative prevalence of each subcategory is demonstrated in Fig. 7A. Most cases had 'SND = OPCA-type' MSA, accounting for almost 50% of our cohort. Of the remaining cases, approximately two-thirds (34% of the total) had 'SND-type' pathology and one-third (17% of total) had 'OPCA-type' pathology. However, the relative frequency of the different pathological phenotypes varies when this is determined in three consecutive, 5 year periods (1988–1992, 1993–1997 and 1998–2002). This shows a gradual decrease in the relative frequency of the SND phenotype while the relative frequency of the OPCA and SND = OPCA phenotypes gradually increased in the same periods (Fig. 7B), which may be due to a change in the recruitment pattern of the QSBB.

Pathological profile in SND- and OPCA-predominant forms

To confirm our pathological criteria for SND and OPCA types, grading of neuronal cell loss in 24 brain sites was compared between 'SND type' and 'OPCA type' using the Mann–Whitney U test (Fig. 8). In the putamen, caudate nucleus and globus pallidus, the neuronal cell loss was always significantly greater in the SND-predominant form than in the OPCA form ($P < 0.0005$), whereas, in the pons, cerebellar hemisphere, cerebellar vermis and inferior olivary nucleus, a greater degree of neuronal cell loss was always observed in the OPCA-predominant form ($P < 0.0005$). As expected, the loss of neuromelanin-containing neurons in most areas of the SbN, namely, SbN1 ($P = 0.08$), SbN2 ($P = 0.007$), SbN4 ($P = 0.02$), SbN5 ($P = 0.001$) and SbN6 ($P < 0.0001$), was greater in the SND-type than in the OPCA-predominant cases (Fig. 8), although it should be noted that the SbN was also severely affected in the OPCA type, in which the mean severity grade for neuronal cell loss was 2.7 in SbN2.

Generally, we found that the grades of frequency of NCIs were 1+ or 2+ in the pontine nuclei, inferior olivary nucleus or the putamen. No NCIs were found in the cerebellar Purkinje cells. NCIs in the putamen were often rounded in shape (Fig. 9A) and sometimes occupied a significant proportion of the cytoplasm, causing indentation of the nucleus (Fig. 9B). Some other NCIs appeared as coiled, perinuclear structures (Fig. 9C). In the pontine nuclei, the majority of the

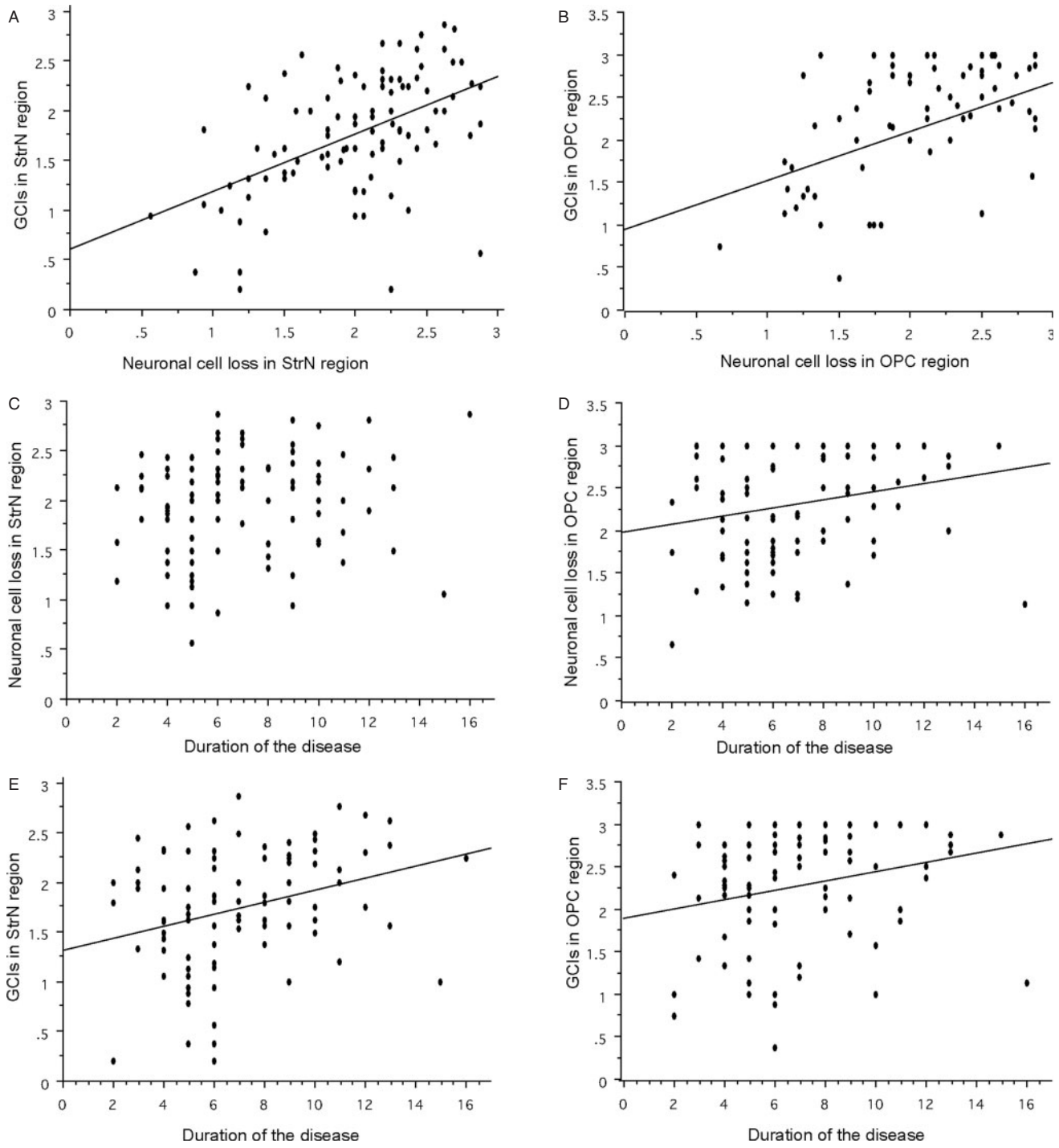

Fig. 5 Relationship between GCIs and neuronal cell loss and between these morphological features and the duration of the disease. A significant correlation was found between the frequency of GCIs and the severity of neuronal cell loss in both the striatonigral (StrN) region ($r = 0.50$, $P < 0.0001$) (**A**) and the olivopontocerebellar (OPC) region ($r = 0.51$, $P < 0.0001$) (**B**). A similar correlation exists between the degree of neuronal cell loss and the duration of the disease in the OPC region ($r = 0.22$, $P < 0.02$) (**D**), but not in the StrN region ($P = 0.07$) (**C**). Conversely, a correlation is found between the frequency of GCIs and the duration of the disease in the StrN region ($r = 0.30$, $P = 0.002$) (**E**); however, such a correlation is weaker in the OPC region ($r = 0.23$, $P = 0.02$) (**F**).

NCIs were round shaped (Fig. 9D), while in the inferior olives NCIs often had irregular outlines and an uneven α-synuclein staining pattern (Fig. 9E). Table 2 shows the percentage of cases that had NCIs in the putamen, pons and inferior olives. There was no difference in the numerical distribution of NCIs within the putamen, pontine nuclei and inferior olivary nuclei between the SND- and OPCA-predominant types.

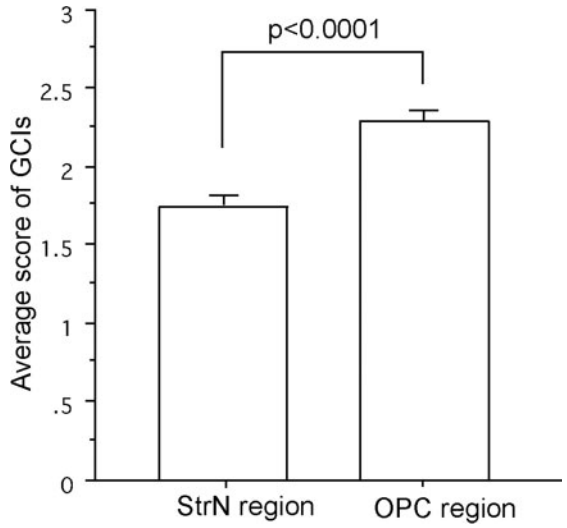

Fig. 6 Comparison of the GCI frequency scores in the striatonigral (StrN) and olivopontocerebellar (OPC) regions. The grading scores of GCIs in the OPC region are significantly higher than those in the StrN region ($P < 0.0001$). Data are represented as mean ± SE.

Table 1 *Pathological phenotypes and grade combinations of pathology*

Pathological phenotype	Grade of StrN pathology	Grade of OPC pathology	Name of combination
SND type	3	2	SND3–OPCA2
	3	1	SND3–OPCA1
	2	1	SND2–OPCA1
OPCA type	2	3	SND2-OPCA3
	1	3	SND1–OPCA3
SND = OPCA type	3	3	SND3–OPCA3
	2	2	SND2–OPCA2

OPC = olivopontocerebellar; OPCA = olivopontocerebellar atrophy; SND = striatonigral degeneration; StrN = striatonigral.

Incidence of Lewy body pathology in MSA

The dorsal motor nucleus of the vagus was available for pathological evaluation in 94 cases. Of these, seven had Lewy bodies both in this nucleus and in the SbN. A further three cases had Lewy bodies exclusively in the dorsal motor nucleus of the vagus, but not in the SbN. These observations indicate that 10.6% of the MSA cases investigated in this study had additional Lewy body pathology (Fig. 9F and G).

Pathological phenotypes and clinical presentation

The clinical diagnosis and gender distribution for each pathological phenotype are provided in Table 3. Analysis of the clinical diagnoses shows that the ante-mortem diagnosis was IPD in a significant proportion of MSA cases irrespective of the pathological subtype (SND type, 50%; OPCA type, 23.5%; SND = OPCA type, 16.3%). A clinical diagnosis of progressive supranuclear palsy (PSP) was made in <10% of our MSA cases (SND type, 8.8%; SND = OPCA type, 4%), all of whom had a grade 3 severity of pathology in the StrN region. Although generally females were slightly over-represented in this series (females : males = 1.2 : 1), 70% of the patients with OPCA type pathology were males. The proportion of males in the OPCA group was significantly higher than that of males in the SND ($P = 0.04$) and SND = OPCA ($P = 0.03$) MSA groups.

Complete clinical data were available for 80 of the 100 cases of MSA referred to the QSBB between 1984 and 2002. Of these 80 cases, 36 (45%) were classified as SND = OPCA pathological phenotype, 31 (38.7%) as SND type and 13 (16.3%) were classified as OPCA type. Comparing the clinical features extracted from the records using the Mann Whitney U test, significant differences in bradykinesia scores and in the number of cases with cerebellar signs were found between the three pathologically defined groups. Initial and final bradykinesia scores were greater in the SND type than in the OPCA type ($P < 0.001$) (Table 4). When comparing the SND = OPCA pathological phenotype with the SND phenotype, the severity of initial, but not final bradykinesia was significantly greater in the SND phenotype ($P < 0.01$) (Table 4). Cerebellar signs were a more common clinical feature in the OPCA pathological phenotype, being present in 61.5% of cases compared with 9.6% of cases with the SND pathological phenotype ($P < 0.001$). Clinical signs or symptoms of autonomic dysfunction and cerebellar signs were found to be more frequent in the SND = OPCA phenotype than the SND phenotype (82.8 and 36.1% of cases, respectively, $P < 0.05$). None of the other clinical features recorded demonstrated any significant difference in frequency or severity between the pathological phenotypes. In particular, there was no difference in the presence or absence of tremor, or the degree of levodopa response, between the pathological phenotypes (Table 4).

With regard to the relationship between the degree of levodopa response and pathological scores in putaminal regions, 84 of the 100 cases had complete data in all four putaminal regions, and 68 cases had complete clinical records with levodopa responsiveness recorded in a way that could be graded. We had both well-documented data on levodopa responsiveness, and complete putaminal pathological data in 57 cases. The mean of the total putaminal pathology scores (for regions Put 1 and Put 2) differed significantly when comparing those with grade 2 and those with grade 3 levodopa response (Fig. 10, $P = 0.03$). There was no significant difference in the mean scores when comparing those with grades 1 and 2 or 1 and 3 levodopa response.

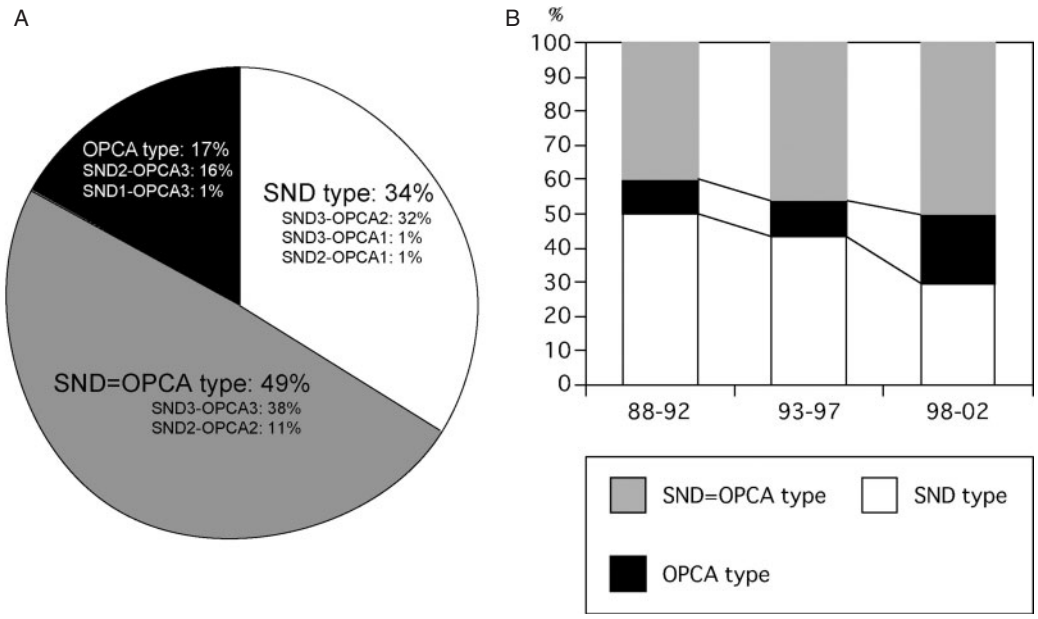

Fig. 7 Distribution of the different pathological subtypes in MSA. Among 100 cases of MSA, the most common category is 'SND = OPCA type' (almost 50% of cases) (**A**). The ratio of phenotypes changed over a 15 year period, with the 'SND type' decreasing while the 'OPCA type' and 'SND = OPCA type' increased gradually during the three consecutive 5 year periods, 1988–1992 (88–92), 1993–1997 (93–97) and 1998–2002 (98–02) (**B**).

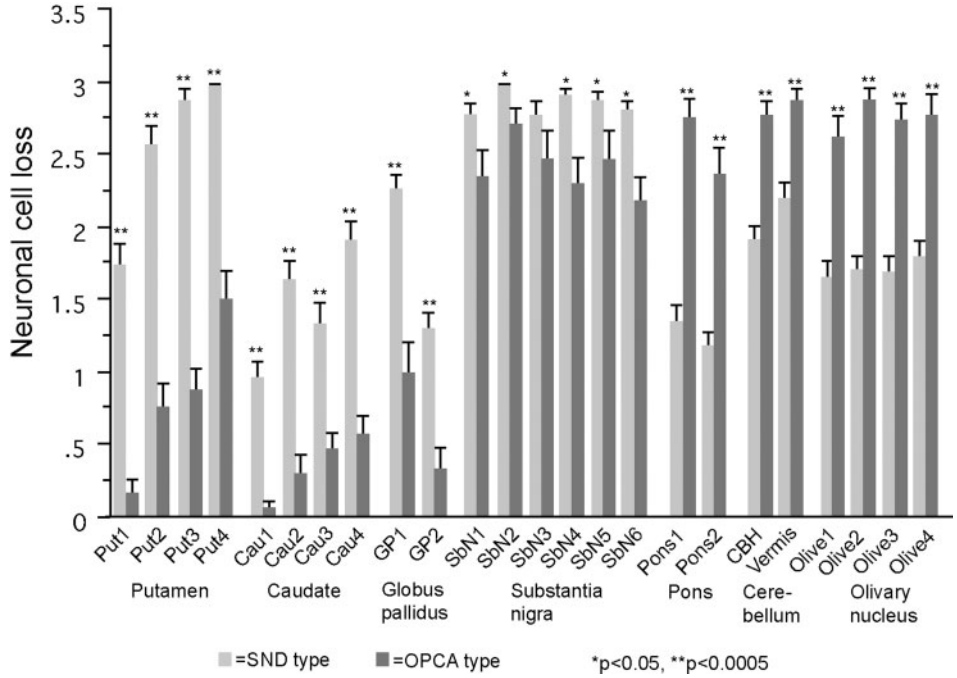

Fig. 8 Comparison of neuronal cell loss between 'SND-type' and 'OPCA-type' cases in the 24 brain sites analysed in the study. 'SND-type' cases have a significantly higher grade of neuronal cell loss in every site of the putamen, caudate nucleus and globus pallidus ($P < 0.0005$), whereas 'OPCA-type' cases have a significantly higher grade of pathology in every site of the pons, cerebellar hemisphere, vermis and olive ($P < 0.0005$). 'SND-type' cases have a higher grade of neuronal cell loss in the substantia nigra (SbN1, SbN2, SbN4, SbN5, SbN6) ($P < 0.05$), except for SbN3 ($P = 0.06$). Data are represented as mean ± SE.

There was no clinical documentation of cerebellar signs in cases with grade 1 severity of pathology in the OPC region, and even in cases with grade 2 severity cerebellar signs were documented in <35% of the cases. On the other hand, parkinsonism was clinically documented in a very high proportion (80%) of cases with grade 1 or 2 severity of pathology in the StrN region. Furthermore, parkinsonism was clinically documented in 87.5% of 'OPCA-type' cases with a grade 1 StrN pathology.

Discussion

Relationship between neuronal cell loss and GCIs

Our semi-quantitative pathological study of 100 MSA cases confirmed that in the StrN region, the SbN and dorsolateral putamen are the most vulnerable sites affected by typical MSA pathology. Given the fact that all six sites of the SbN were severely affected, it is possible that neurodegeneration occurs first in the SbN, and then involves the dorsolateral part of the putamen and subsequently other sites of the basal ganglia. With regard to OPCA pathology, the cerebellar vermis appeared the most vulnerable anatomical structure; however, as all OPC sites had a mean severity grade greater than 2+, the relative vulnerability of each individual site was difficult to ascertain.

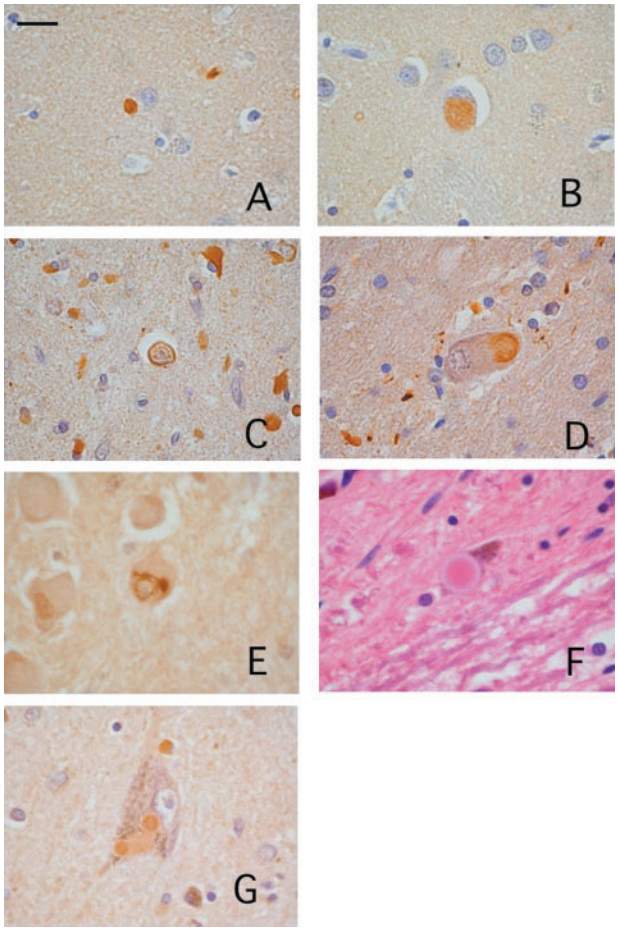

Fig. 9 Examples of neuronal cytoplasmic inclusions (NCIs) in the putamen, pontine nuclei and inferior olivary nucleus, as well as Lewy bodies in the substantia nigra and dorsal motor nucleus of the vagus in MSA. (**A**) A rounded NCI in a neuron of the putamen. Some of the NCIs are large and indent the nucleus (**B**), others are ring-like and have a perinuclear distribution (**C**). (**D**) In the pontine nucleus, typical round-shaped NCIs are common. (**E**) An irregular, partly perinuclear inclusion in the inferior olivary nucleus. Lewy bodies are seen in the substantia nigra (**F**) and the dorsal motor nucleus of the vagus (**G**) in a relatively small number of cases. **F**, H&E staining; **A–E** and **G**, α-synuclein immunohistochemistry. The scale bar represents 20 μm.

We found a significant correlation between the density of GCIs and the severity of neuronal cell loss in both the StrN and OPC regions, which indicates that GCIs are closely associated with the neurodegenerative process in MSA. Oligodendrocytes provide neurons with trophic factors (Du and Dreyfus, 2002) and/or other signal molecules (Fields and Stevens-Graham, 2002) which are essential for normal neuronal function and survival. It is possible that oligodendroglial α-synuclein aggregates, morphologically manifesting as filamentous inclusions (GCIs), are able to compromise normal oligodendroglial functions and that this process results not only in oligodendroglial, but also in neuronal dysfunction (Wenning *et al*., 1994*b*) and ultimately cell death in vulnerable regions of the CNS in MSA. The primary importance of oligodendroglial dysfunction in the neurodegenerative process of MSA may also be supported by the observation that the distribution of cells undergoing apoptosis, which is a form of programmed cell death, is comparable with that of the GCI pathology and also that apoptosis primarily affects oligodendroglial cells in MSA (Probst-Cousin *et al*., 1998).

Do GCIs always contribute to neuronal cell loss in every vulnerable region in MSA? With regard to vulnerability of the SbN, a recent report demonstrated that neurons in the SbN were highly vulnerable to the toxicity of α-synuclein (Feany and Bender, 2000); furthermore, overexpression of neuronal α-synuclein can cause rapid neuronal cell loss in the SbN *in vivo* (Lo Bianco *et al*., 2002). These results indicate that accumulation of α-synuclein can primarily damage neurons in the SbN. In fact, our semi-quantitative pathological study and a novel pathological index 'NCLPS' in the SbN demonstrated that neuronal cell loss was always severe even when the appearance of GCIs was mild, indicating that the neurodegenerative process in the SbN was not simply influenced by oligodendroglial α-synuclein aggregates. These results suggest that in the SbN, the cell death mechanisms may exist primarily in the neurons themselves. Thus, although the number of GCIs also gradually increases with

Table 2 *Incidence of NCIs in putamen, pons and olivary nucleus*

Pathological phenotype	Put 1	Put 2	Put 3	Put 4	Pons 1	Pons 2	Olive 1	Olive 2	Olive 3	Olive 4
SND type	37.9%	44.8%	36.7%	56.7%	70.0%	66.7%	76.7%	57.7%	84.6%	54.5%
OPCA type	23.5%	23.5%	43.8%	43.8%	81.3%	87.5%	87.5%	53.3%	80.0%	44.4%
SND = OPCA type	32.5%	45.0%	45.5%	50.0%	76.7%	86.0%	82.9%	38.2%	87.5%	53.1%

NCIs = neuronal cytoplasmic inclusions; put = putamen.

Table 3 *Gender distribution, age at onset and clinical diagnosis*

Pathological phenotype	No. of males : females	Age at onset (years) mean (range)	Clinical diagnosis (%)			
			MSA	IPD	PSP	Others
SND type	14 : 20	61.8 (44–79)	35.2	50**	8.8	5.8
OPCA type	12 : 5*	54.8 (37–77)	76.4	23.5	0	0
SND = OPCA type	20 : 29	55.5 (34–83)	73.4	16.3	4.0	8.1

MSA = multiple system atrophy; IPD = idiopathic parkinson's disease; PSP = progressive supranuclear palsy. *P = 0.04; **P = 0.0009 (risk of false-positive diagnosis of IPD if SND type).

Table 4 *Pathological phenotype and clinical features of parkinsonism*

Pathological phenotype	Initial bradykinesia score (mean ± SD)	Final bradykinesia score (mean ± SD)	Total score (mean ± SD)	Levodopa response score (mean ± SD)
SND type	1.5 ± 0.8 (n = 31)	2.5 ± 0.7 (n = 31)	4.0 ± 1.4 (n = 31)	1.7 ± 0.7 (n = 30)
OPCA type	0.5 ± 0.7*** (n = 13)	1.2 ± 1.0*** (n = 13)	1.6 ± 1.5*** (n = 13)	2.0 ± 0.8 (n = 7)
SND = OPCA type	0.8 ± 0.8** (n = 36)	2.4 ± 0.8$^{\#\#\#}$ (n = 36)	3.2 ± 1.4$^{*,\#\#}$ (n = 34)	1.5 ± 0.8 (n = 34)

Bradykinesia score; 0 = none, 1 = mild, 2 = moderate, 3 = severe. Total score is the sum of the initial and final scores. Levodopa response is graded on a 4-point scale 1 = none/minimal, 2 = moderate, 3 = good and 4 = excellent. Scores are compared using the Mann–Whitney U test (SND type versus others; *P < 0.05, **P < 0.01, ***P < 0.001: OPCA vs SND = OPCA; $^{\#\#}P$ < 0.005, $^{\#\#\#}P$ < 0.001).

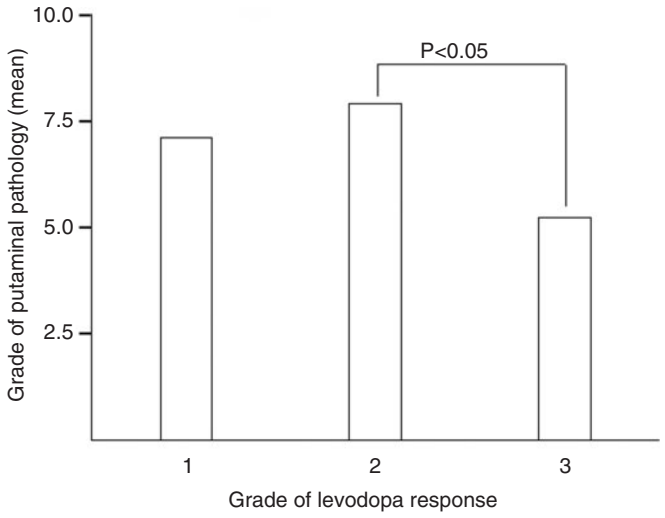

Fig. 10 Relationship between the severity of putaminal pathology and levodopa response. Cases with good levodopa response (grade 3) are associated with less severe putaminal pathological changes. None of the cases had an excellent levodopa response (grade 4) in this series of 100 MSA cases. Mean total pathology scores for putaminal regions 1 and 2 (severity of GCI plus severity of neuronal loss graded as 0 = none, 1 = mild, 2 = moderate, 3 = severe for each region) grouped according to recorded levodopa responsiveness (1 = no or a minimal response, 2 = a moderate response, 3 = a good response and 4 = an excellent response to levodopa).

time in the StrN region, neuronal loss occurs sooner and may take place in a different manner in this anatomical area. Given the fact that NCLPS of the SbN was extremely high, our data strongly suggest that neuronal loss takes place in a rapid manner in the StrN region. Furthermore, we found that the density of GCI in the StrN region was significantly lower than that in the OPC region. Taken together, these observations raise the possibility that GCIs contribute less to the pathogenesis of neurodegeneration in the StrN than they do in the OPC region, and also that factors other than GCIs contribute to the neurodegenerative process in the StrN region.

The spectrum of pathological phenotypes of MSA

This study has confirmed the sensitivity of our microscopic criteria for establishing different MSA pathological phenotypes. This is supported by our findings confirming the presence of more severe pathological changes in the StrN region than in the OPC area in the 'SND-type' cases and, conversely, a predominance of the morphological changes in the OPC area over the StrN region in cases with 'OPCA-type' MSA. With regard to the nosology of MSA, our results clearly indicate that there is a wide spectrum of severity of the pathological changes in MSA giving rise to the 'SND', 'OPCA' and 'SND = OPCA' types of MSA, with the majority morphologically belonging to the 'SND = OPCA' type. Although our finding of a difference in gender distribution between the OPCA and SND types raises the possibility of additional biological factors determining this differential emphasis of pathological changes, we interpret this observation with caution, as the sample size of our study was relatively small, and this finding should be validated by future studies. We failed to identify any cases with pure SND or OPCA pathology in this series, indicating that it is likely that neither StrN nor OPC pathology exists in isolation.

In our series of 100 MSA cases, the relative frequency of cases with predominant OPC pathology has gradually increased since 1988. Clinical criteria, which improve clinical diagnostic accuracy in MSA (Osaki *et al*., 2002), have only been established in recent years (Quinn, 1989; Gilman *et al*., 1999) after the discovery of GCIs as a hallmark lesion (Papp *et al*., 1989). These may have raised awareness of clinical subtypes with ataxia, and might explain why these cases with ataxia subsequently were more likely to be recruited for ultimate brain autopsy. Our observation that the relative frequency of OPCA has increased in our pathological series of MSA cases may also have implications on the validity of previous observations suggesting that SND is relatively more frequent, and OPCA less frequent among Caucasians than in the Japanese population (Wenning *et al*., 1994*a*; Watanabe *et al*., 2002), and points to the need for future comparative morphological studies using a similar approach to ours. However, in relation to the ratio of SND and OPCA cases, selection bias is also a crucial factor. Our brain bank was for many years dedicated to parkinsonian disorders, which still remains its principal material, whereas others may be attached to centres with a particular interest in cerebellar disorders.

NCIs and Lewy bodies in MSA

The structures that previously have been described to contain NCIs in MSA include the pontine nuclei and inferior olivary nuclei (Arima *et al*., 1998; Yokoyama *et al*., 2001; Sakurai *et al*., 2002). In our series, there was no difference between SND- and OPCA-type pathology in the frequency of NCIs within the putamen, pontine nuclei and inferior olives, suggesting that NCIs may not relate to the severity of neuronal cell loss. Although Purkinje cell loss is invariable in MSA, we did not detect NCIs in this type of neuron. This finding is in keeping with the observation that α-synuclein does not accumulate in Purkinje cells (Mori *et al*., 2003).

The frequency of Lewy bodies has been reported to be 12.5% in PSP (mean age 73 years) and 9.2% in control brains (mean age 78 years) (Tsuboi *et al*., 2001). Although the mean age of the MSA cases used in the current study (64 years) was lower, the 10.6% frequency of Lewy bodies in MSA is similar to that in PSP and control brains.

Clinicopathological correlations

Clinical features reflect the pathological phenotype

There is a significant difference in the severity of bradykinesia and the presence of cerebellar signs between the pathological phenotypes of MSA. The SND type demonstrates the most severe bradykinesia and the OPCA type the more frequent occurrence of cerebellar signs, indicating that the clinical phenotype is dependent on the distribution of pathology within the basal ganglia and cerebellum. Therefore, our results verify the presumption that MSA-P has a predominantly StrN, and MSA-C a predominantly OPC pathology.

Levodopa responsiveness and putaminal pathology

An important pointer to a diagnosis of MSA is a response to levodopa that is poor (Wenning *et al*., 2000), or unsustained (Hughes *et al*., 1992), in contrast to the marked long-term response to levodopa characteristic of IPD (Barbeau and Roy, 1976). Indeed, of the 68 cases in this series who had a trial of levodopa, 31 reported a minimal, 25 a moderate, only 12 a good and none an excellent response to levodopa. Considering the different pathological involvement in the StrN region between MSA and IPD, it has been hypothesized that putaminal pathology is responsible for the poor levodopa response in MSA (Fearnley and Lees, 1990). Our results demonstrate that the anterior part of the putamen (Put 1 and Put 2) is preserved in cases with good response to levodopa. Since the pathological involvement of the putamen starts dorsally and, as it progresses, spreads ventrally and anteriorly in a dorsolateral fashion, our observations suggest that the tempo of this spread is slow and the pathological changes relatively mild in good levodopa responders. Although there are difficulties in making assumptions regarding levodopa responsiveness in MSA using post-mortem pathological findings, our observations nevertheless strongly suggest that the extent of putaminal involvement determines the poor levodopa response which is a characteristic clinical feature of most MSA cases.

Parkinsonism versus cerebellar signs in MSA

Why was there a high rate of misdiagnosis as IPD even in individuals with predominant OPCA pathology? One of the reasons is the significant involvement of the SbN occurring in OPCA-type MSA. Another observation is that parkinsonian symptoms and signs were present even in cases in which the severity of StrN pathology was only grade 1. This observation indicates that 1+ or 2+ neuronal cell loss in SbN can be associated with clinically manifest parkinsonism in the MSA cases, although it has been suggested that by the time symptoms begin, ∼50% of neurons are lost in the caudal SbN in IPD (Fearnley and Lees, 1991). However, our study also demonstrated that, irrespective of the grade of nigral pathology, this is always accompanied by putaminal involvement, so that the combination of these two pathologies results in clinically obvious parkinsonism even in cases with relatively mild involvement of the SbN. In contrast, a severity grade of $\geqslant 2$ was required for cerebellar signs to be clinically documented, in keeping with the observation that the frequency of cerebellar signs in MSA was low even in the cases with obvious cerebellar involvement at autopsy (Wenning *et al*., 1995, 1996). These results may indicate that the level of StrN pathology required for the clinical manifestation of a parkinsonian syndrome is relatively less

than the corresponding OPC pathology for the manifestation of cerebellar signs.

Conclusions

In this study, we performed a semi-quantitative analysis of pathological changes in both StrN and OPC regions in a large cohort of MSA brains (100 cases). A spectrum of pathological phenotypes of MSA was detected. Based on the dynamic relationship between the density of GCIs and severity of neuronal cell loss, as well as between the pathology and the disease duration at death, our data raise the possibility that the disease process in 'OPCA-type' MSA may have a different course from that seen in 'SND-type' MSA. In particular, we have demonstrated that GCIs contribute more to the pathogenesis of OPC pathology than to StrN pathology. We also conclude that Lewy body pathology is unlikely to be a primary process in MSA.

At a clinicopathological level, the SND phenotype demonstrates the most severe bradykinesia and the OPCA phenotype the more frequent occurrence of cerebellar signs, indicating that the clinical phenotype is dependent on the distribution of pathology within the basal ganglia and cerebellum. The hypothesis that putaminal pathology is responsible for a poor levodopa response in MSA has been confirmed in this study. Our data also suggest that the pathological severity threshold for clinical parkinsonism is lower than that for ataxia, which may explain why parkinsonism usually predominates over ataxia in MSA.

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