RESEARCH ARTICLE

Neuropathology with Clinical Correlations of Sporadic Amyotrophic Lateral Sclerosis: 102 Autopsy Cases Examined Between 1962 and 2000

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Sporadic amyotrophic lateral sclerosis (ALS) is a fatal neurological disorder affecting adults. We studied the neuropathology and clinical correlations in 102 autopsy cases of ALS. The age at onset of the disease was significantly higher for the bulbar-onset form (30 cases) than for the limb-onset form (72 cases). Dementia was confirmed in 7 cases. These 102 cases were divided into 4 pathological subgroups: typical ALS (59 cases), lower-motor-predominant ALS (23 cases), ALS with temporal lesions (18 cases), and ALS with pallido-nigro-luysian degeneration (2 cases). The age at onset was significantly higher for lower-motor-predominant ALS and ALS with temporal lesions than for typical ALS. In the lower motor neurons, Bunina bodies were detected in 88 cases, whereas ubiquitin-immunoreactive skein and/or spherical inclusions were detected in all 102 cases. Of the 100 available cases, 50 and 16 also showed ubiquitin-immunoreactive inclusions in the neostriatal and temporal small neurons, respectively. Ubiquitin-immunoreactive dystrophic neurites were also observed in the neostriatum in 3 of the 50 cases with neostriatal inclusions, and in the temporal cortex in 4 of the 16 cases with temporal inclusions. There was a significant association between the bulbar-onset form, temporal lesions, neostriatal inclusions and temporal inclusions, and between dementia, temporal lesions and temporal inclusions. Neostriatal and temporal dystrophic neurites were associated with dementia and bulbar-onset form through temporal lesions and temporal inclusions. The present findings may be helpful for designing further studies on the mechanisms underlying the development of ALS.

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Introduction

Sporadic amyotrophic lateral sclerosis (ALS) is a progressive neurological disorder of unknown cause that occurs in adults. The prevalence of ALS in the United States in 2000 was estimated to be approximately 7 per 100 000 population (56). Several recent epidemiological studies have identified an increase in the frequency of occurrence of ALS, especially in the aged population(36, 47-49). The occasional association between this disease and frontal lobe dysfunction (1) and dementia (15, 38) is also well described.

Neuropathologically, degeneration of both the upper and lower motor neuron systems is the principal, essential pathological feature of ALS. However, there is accumulating evidence that the neurodegenerative process is also present in the non-motor neuron system, including the limbic system, basal ganglia, and intermediate zone of the spinal cord (16, 30). Moreover, degenerative lesions may also be found in various brain regions other than the motor neuron system in some patients who had been on a respirator for long periods (12, 74). With regard to the cellular pathology of ALS, it is of great importance that in this disease, characteristic neuronal cytoplasmic inclusions (ie, Bunina bodies [53, 70] and ubiquitinated inclusions [22, 28, 32]) have been described in the lower motor neurons. At present, however, compared with the neurofibrillary tangles in Alzheimer's disease, Lewy bodies in Parkinson's disease, or neuronal intranuclear inclusions in polyglutamine diseases, much less is known about the molecular profiles of the inclusions associated with ALS (10).

At the Department of Pathology, Brain Research Institute, Niigata University, Japan, 102 clinicopathologically established cases of sporadic ALS, including 28 who had had artificial respiratory support (ARS), were seen from 1962 through 2000. The present study addresses the following issues with regard to ALS: *i)* the

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Subgroup	No. of cases (Male/Female)	Age at onset * (years)	Age at death * (years)	Disease duration † (months)	ARS duration † (months)
Without-ARS	74 (35/39)	60.2±12.6 (27-86)	63.5±12.2 (31-87)	25.0 (7-240)	0
With-ARS	28 (20/8)	58.6±9.2 (40-81)	64.6±8.1 (48-82)	53.5 (12-168) ‡	30 (5-156)
Total	102 (55/47)	59.8±11.7 (27-86)	63.8±11.2 (31-87)	33.0 (7-240)	

* The data are presented as the mean ± S.D. (range).
† The data are presented as the median (range).
‡ p=0.0002 (Mann-Whitney *U*-test), comparison with the without-ARS data.

Table 1. Clinical findings in subgroups without and with artificial respiratory support (ARS) among 102 cases of sporadic amyotrophic lateral sclerosis (ALS).

Onset form		No. of cases (Male/Female)	Age at onset * (years)	Age at death * (years)	Disease duration † (months)
Bulbar	ARS (-)	23 (9/14)	64.2±11.7 ‡	66.4±11.8	20.0 (7-156) §
	ARS (+)	7 (3/4)		68.6±6.3	53.0 (12-149)
Limb	ARS (-)	51 (26/25)	57.9±11.3	62.1±12.2	32.0 (9-240)
	ARS (+)	21 (17/4)		63.2±8.3	54.0 (17-168)

* The data are presented as the mean±S.D.
† The data are presented as the median (range).
‡ p=0.0134 (Student's t-test)
§ p=0.0192 (Mann-Whitney U-test), comparison with the limb-onset form

Table 2. Clinical findings in bulbar- and limb-onset forms among 102 cases of sporadic ALS.

clinical findings, such as the age at onset, disease duration and initial symptoms, *ii)* the severity and distribution pattern of histological involvement of the motor and non-motor neuron systems, and *iii)* the occurrence of neuronal inclusion bodies (Bunina bodies and ubiquitinated inclusions). The clinical and neuropathological data obtained were analyzed statistically.

Materials and Methods

Subjects. The autopsy ledger at our department was searched for all clinicopathologically confirmed cases of ALS that were logged between 1962 and 2000. A total of 102 cases of sporadic ALS were seen during this period. In all of the cases, the clinical and pathological diagnoses were made without any major differential problems: *i)* there were no family histories of similar neurological disorders, *ii)* motor weakness, dyspnea or dysphagia appeared as the initial symptoms (in one case, dementia was noted as the initial symptom), and *iii)* the pathological examinations confirmed neuronal degeneration and loss in both the upper and lower motor neuron systems. Some unusual cases have already been reported in detail (18, 19, 21, 77). A case of atypical ALS with multisystem degeneration, in which skein inclusions (confirmed later in the anterior horn cells) and Bunina bodies were observed, was excluded from the present study, because the patient's parents were first cousins (67).

Autopsy records and postmortem materials. The autopsy file for each case contained the clinical and pathological data and the lists of tissue samples taken, including those for light and electron microscopic observations. Color slides prepared for multiple macroscopic and light-microscopic analysis, as well as multiple histological specimens and the original formalin-fixed, paraffin-embedded tissue blocks were available in all cases. In addition, specimens of ubiquitin-immunostained sections containing the lower motor neurons of the spinal cord and brainstem (in most cases, motor nucleus V, the facial nucleus and hypoglossal nucleus were included) were available in cases logged after 1990.

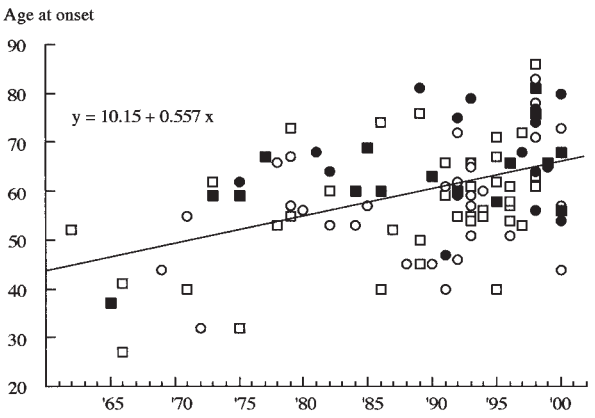

Figure 1. The age at onset of sporadic amyotrophic lateral sclerosis (ALS) for 102 cases is plotted against years from 1962 through 2000. The age at onset tended to rise year after year ($r = 0.449$, $p<0.0001$). Solid squares (men) and circles (women) represent a total of 32 individuals affected pathologically by lower-motor-predominant ALS.

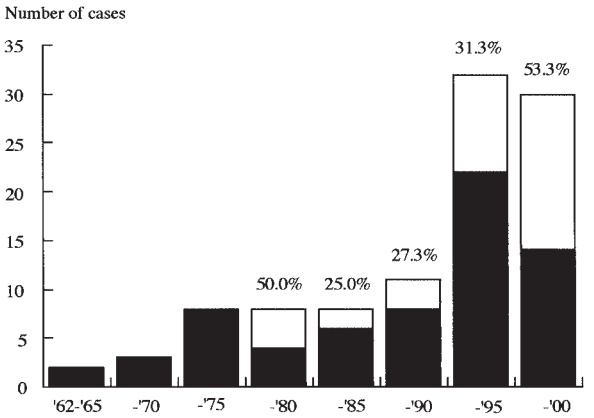

Figure 2. The frequency of 102 cases of sporadic ALS expressed in terms of 5-year intervals from 1966 through 2000, and for the 4-year interval 1962 to 1965. The stacked empty bars show the contribution to the case numbers by cases >65 years old at disease onset.

Subgroup		No. of cases (Male/Female)	Age at onset * (years)	Age at death * (years)	Disease duration † (months)
Typical ALS	ARS (-)	39 (19/20)	55.9±11.1	58.6±12.0	33.0 (11-156)
	ARS (+)	20 (15/5)		63.6±7.0	54.5 (17-168)
Lower motor-predominant ALS	ARS (-)	18 (9/9)	68.0±7.5 ‡	71.5±7.0 ‖	21.0 (7-240)
	ARS (+)	5 (4/1)		72.2±7.6	26.0 (12-54)
ALS with temporal lesions	ARS (-)	16 (7/9)	62.9±12.4 §	66.1±12.4 ¶	20.5 (10-64) #
	ARS (+)	2 (1/1)		64/63	120/149
ALS with PNL degeneration	ARS (-)	1 (0/1)	54	67	156
	ARS (+)	1 (0/1)	45	48	33

PNL: pallido-nigro-luysian.
* The data are presented as the mean±S.D.
† The data are presented as the median (range).
‡ $p<0.0001$, § $p=0.0255$, ‖ $p<0.0001$ and ¶ $p=0.0433$ (Student's t-test), comparison with typical ALS.
# $p=0.0144$ (Mann-Whitney U-test), comparison with typical ALS.

Table 3. Clinical findings in pathological subgroups among 102 cases of sporadic ALS.

Electron micrographs showing neuronal inclusion bodies were also available in several cases. We reviewed these autopsy files and postmortem materials. When necessary for the present study, we prepared new 4-μm-thick, paraffin-embedded sections from the original blocks of the motor cortex (usually cut transversely at four different levels along the long axis), spinal cord (usually cut transversely at the levels of C7, T8, L4 and S2-3), basal ganglia or temporal lobe. These sections were stained with hematoxylin and eosin, Klüver-Barrera, or immunostained with a polyclonal antibody against ubiquitin (Dako, Glostrup, Denmark; 1:800), a monoclonal antibody against phosphorylation-dependent tau (AT8; Innogenetics, Ghent, Belgium; 1:200) or a polyclonal antibody against α-synuclein (NACP-5 (78); 1:2000), using the avidin-biotin-peroxidase complex method with the diaminobenzidine as the chromogen.

Conventional electron microscopy and immunoelectron microscopy. In the present study, Bunina bodies and skein inclusions in the spinal anterior horn neurons were examined ultrastructually in one selected

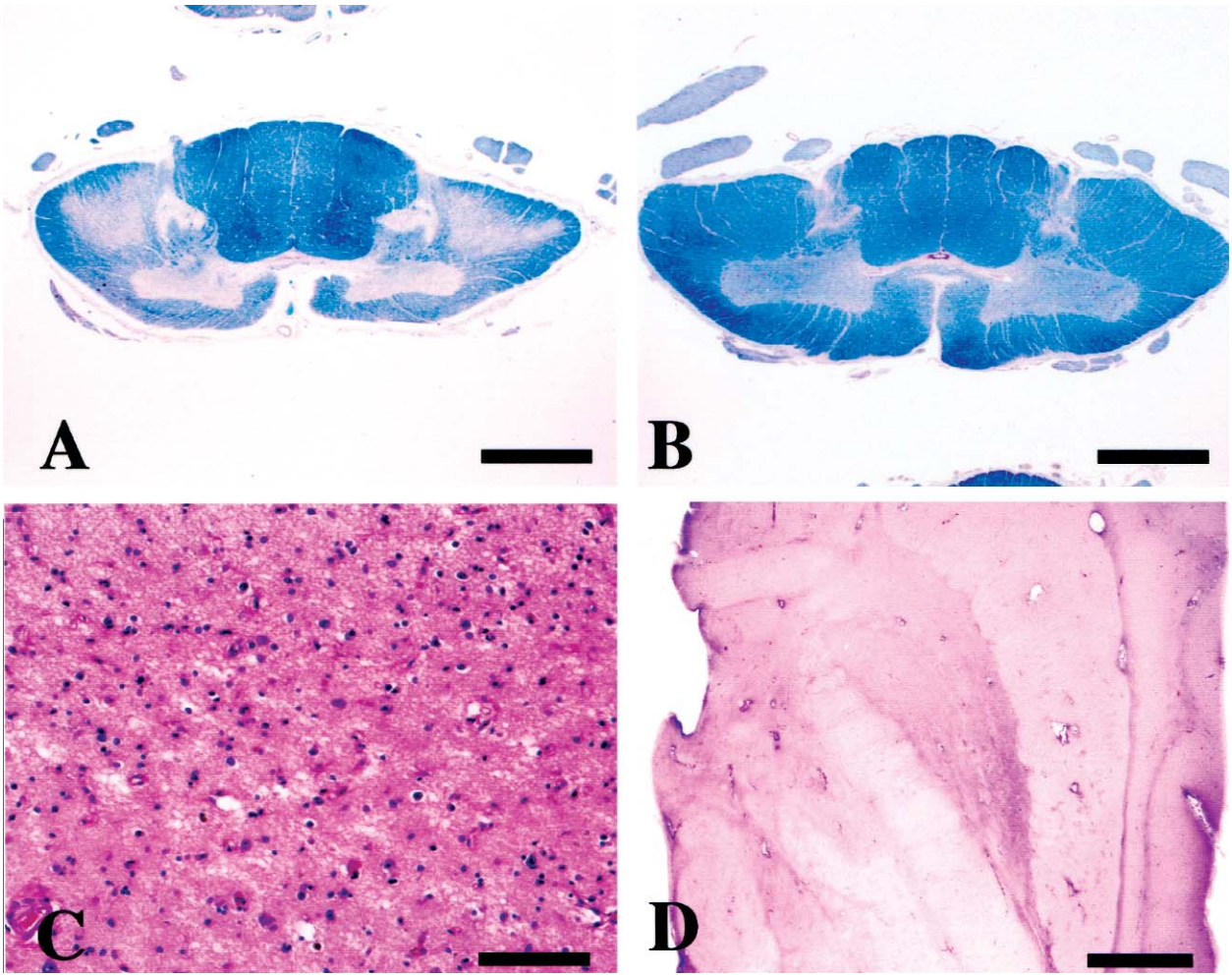

Figure 3. (**A, B**) Long tract degeneration of sporadic ALS. Myelin pallor of the lateral corticospinal tract is evident in (**A**), but not in (**B**). Cervical cord (C7), Klüver-Barrera. Bars = 2 mm. (**C**) Neuronal loss, astrocytic gliosis and vacuolization are seen in the medial cortex (layers II and III) of the temporal tip. Hematoxylin and eosin (H&E). Bar = 100 μm. (**D**) Fibrillary astrocytic gliosis is evident in the horizontally cut globus pallidus. Holzer. Bar = 5 mm.

case. In addition, 50-μm-thick vibratome sections from the putamen of another selected case were prepared for immunoelectron microscopy: the ultrastructural localization of ubiquitin was examined using a pre-embedding method with the above-mentioned anti-ubiquitin antibody (1:800), as described previously (76).

Statistical analysis. We analyzed the 102 autopsy cases statistically. Differences between the variances were tested by Student's *t*-test, or by the Mann-Whitney *U*-test as a non-parametric (distribution-free) method. Associations between the two groups were assessed by the chi-squared test. Calculations were performed using commercial software, StatView (Abacus Concepts, Berkeley, Calif), on an Apple Macintosh personal computer. The level of statistical significance was set at $p<0.05$.

Results

Clinical features. The clinical features are summarized in Tables 1 and 2. Among the 102 ALS cases, with- and without-ARS subgroups, and bulbar- and limb-onset (upper = 40, upper and lower = 4, lower = 28) forms were recognized. In 35 cases (34.3%) onset occurred at >65 years of age (Figure 1), and the percentage of such cases was 53.3% in the last 5-year interval (Figure 2). When data from only the without-ARS subgroup were used for analysis, the disease duration in cases for which the age at onset was >65 years (30 cases: median

Subgroup	No. of cases (Male / Female)	Bunina body	Motor neuron inclusion	Ubiquitinated Neostriatal inclusion [neurite] *	Temporal inclusion [neurite] *
Typical ALS	59 (34/25)	49/59 (83.1%)	59/59 (100%)	28 [1]/57 (49.1%)	3 [1]/57 (5.3%)
Lower motor-predominant ALS	23 (13/10)	22/23 (95.7%)	23/23 (100%)	7 [1]/23 (30.4%)	2 [0]/23 (8.7%)
ALS with temporal lesions	18 (8/10)	15/18 (83.3%)	18/18 (100%)	14 [1]/18 (77.8%)	11 [3]/18 (61.1%)
ALS with PNL degeneration	2 (0/2)	2/2 (100%)	2/2 (100%)	1 [0]/2 (50.0%)	0 [0]/2 (0%)
Total	102 (55/47)	88/102 (86.3%)	102/102 (100%)	50 [3]/100 (50.0%)	16 [4]/100 (16.0%)

These neuronal inclusions were immunonegative for both tau and α-synuclein. * A total of 100 cases were available for study.

Table 4. The frequency of neuronal inclusion bodies in pathological subgroups among 102 cases of sporadic ALS.

19 months, range 9-60 months) was significantly shorter than for the remainder (44 cases: median 41 months, range 7 months to 20 years; $p = 0.0002$).

In addition to the symptoms of motor neuron disturbance, dementia was confirmed in 7 cases (6.9%; male/female = 3/4, bulbar-onset/limb-onset = 4/3), where the age at ALS onset was between 37 and 66 years (mean 57.7 years). In these 7 cases, no tau or α-synuclein pathology that might have contributed to the manifestations of dementia was observed (data not shown), and generally, the dementia was relatively mild in degree.

Eighteen patients (17.6%) had a history of external injury. Most of these were bone fractures, and the affected sites varied from case to case, including the limbs (8 cases) and spine (3 cases).

Spondylotic myelopathy was confirmed at autopsy in six cases (5.9%; male/female = 3/3, cervical/lumbar = 5/1), regardless of the presence or absence of a clinical history. One patient also had a history of external injury. The age at ALS onset for these six cases (mean 69.8 years, range 53-81 years) was significantly higher than for the remainder ($p = 0.0293$).

Pathological subgroups. We were able to divide the 102 cases into 4 subgroups based on the histological findings from the upper motor neuron and non-motor neuron systems (Table 3). In 82 of the 102 ALS cases, degenerative changes were essentially confined to the upper and lower motor neuron systems (for convenience, 2 exceptional cases, one with ubiquitinated intranuclear inclusions in the hippocampal pyramidal neurons [21] and the other with severe sensory neuronopathy [77], were included here). These cases were divided into 2 subgroups: typical ALS (59 cases) and lower-motor-predominant ALS (23 cases). In cases of typical ALS, neuronal loss and astrocytic gliosis in both the motor cortex and spinal anterior horn were evident, as well as myelin pallor in the corticospinal tract (Figure 3A). In lower motor-predominant ALS, only mild loss of Betz cells in the motor cortex (the holes containing lipofuscin-laden macrophages, from which Betz cells had presumably disappeared, were detected among the remaining, often normal-looking Betz cells) and no remarkable myelin pallor in the corticospinal tract were observed (only a small number of sudanophilic droplets were detected in the perivascular regions in the lateral column) (Figure 3B). In more than half of the 23 cases of lower-motor-predominant ALS (14 cases), the age at onset was >65 years.

In addition to motor neuron involvement, obvious neuronal loss and astrocytic gliosis in the medial cortex of the temporal tip, and/or the CA1-subiculum transitional zone in the pes hippocampi were observed in 18 cases, representing a subgroup of ALS with temporal lesions (Figure 3C). Of these 18 cases, 9 also showed mild, but detectable fronto-temporal atrophy, and obvious degeneration of the substantia nigra: dementia was present in 6 of those 9 cases. Of the 18 cases of ALS with temporal lesions, 8 showed no remarkable myelin pallor in the corticospinal tract.

Similarly, obvious neuronal loss and astrocytic gliosis in the globus pallidus, substantia nigra and subthal-

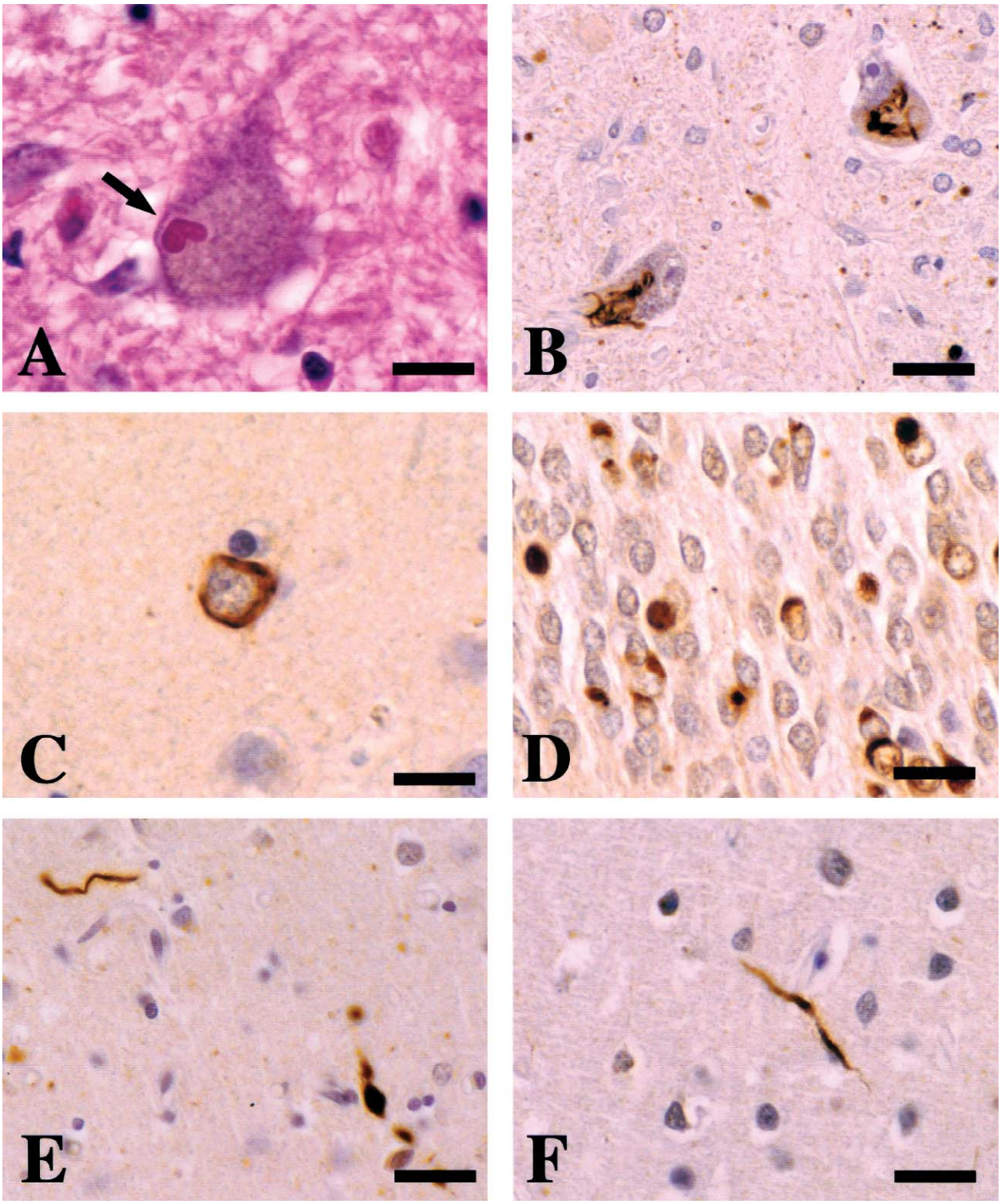

Figure 4. Neuronal cytoplasmic inclusion bodies and dystrophic neurites. (**A**) Small beaded eosinophilic structures (Bunina bodies; arrow) in a lipofuscin-rich hypoglossal nucleus neuron. Note that Bunina bodies are not homogenous structures. (**B-D**) Skein inclusions in two spinal anterior horn neurons (**B**), a circular inclusion in a putaminal small neuron (**C**), and round- or crescent-shaped inclusions in some dentate granule cells (**D**). (**E, F**) Dystrophic neurites in the putamen (**E**) and the second layer of the parahippocampal gyrus (**F**). (**A**) H&E; (**B-F**) Anti-ubiquitin, diaminobenzidine/hematoxylin. Bars = 12.5 μm for **A** and **C**; 25 μm for **B, D, E, F**.

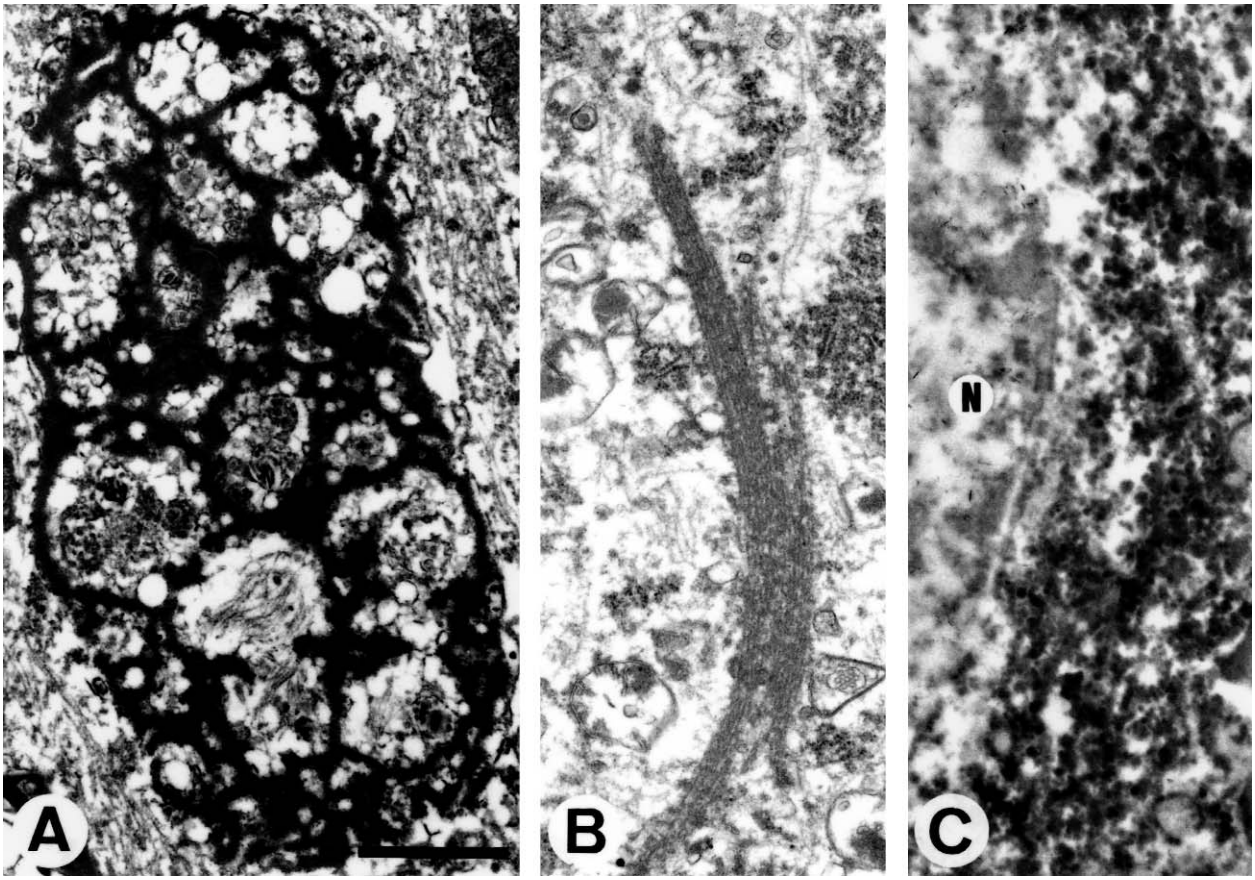

Figure 5. Ultrastructures of neuronal cytoplasmic inclusions. (**A**) A Bunina body, showing electron-dense material containing many vesicular structures, as well as accumulations of neurofilaments (lower). (**B**) A skein inclusion, showing bundles of unconstricted filaments, about 15 to 25 nm in diameter. (**C**) A ubiquitin-labeled putaminal inclusion, showing granular and partly filamentous structures. N; nucleus. Bar = 1 μm for **A, B, C**.

amic nucleus were observed in 2 cases, representing ALS with pallido-nigro-luysian (PNL) degeneration (Figure 3D). No temporal lesions (including tau pathology) were observed in these cases. One case, with a disease duration of 13 years, showed no obvious myelin pallor in the corticospinal tract. Of interest was that these 2 cases were from the same, small district in Niigata Prefecture. There were no consanguineous marriages in these families.

Bunina bodies and ubiquitin-immunoreactive inclusions in lower motor neurons. Bunina bodies may also be found in the upper motor neurons (61) and nonmotor neurons in ALS (19, 44, 54, 67, 69). Ubiquitin-immunoreactive neuronal inclusions in the motor cortex are also associated with ALS (29, 31). However, in each case, our present investigations of these neuronal inclusion bodies were limited to the lower motor neurons in the spinal cord and brainstem. The results are shown in Table 4.

Bunina bodies are defined as small (several micrometers in diameter), round eosinophilic neuronal cytoplasmic inclusions (33, 53, 64, 70) (Figure 4A). Routine histopathological examinations revealed that in 18 cases exhibiting very severe loss of lower motor neurons, Bunina bodies were not detected. However, they were observed subsequently in 4 of those 18 cases following further observations of new serial sections. Some studies have shown that Bunina bodies are ubiquitin-immunoreactive (32, 40). However, our present immunohistochemical examinations revealed no positive staining that might correspond to Bunina bodies. On the other hand, the presence of cystatin C-immunoreactive Bunina bodies (50) was confirmed (data not shown). The disease duration for cases exhibiting Bunina bodies was significantly shorter than for those without them ($p = 0.0016$). There was no significant difference

in the age at onset between those with and those without.

Here, ubiquitin-immunoreactive inclusions are defined as so-called ubiquitinated skein or spherical structures within the neuronal cytoplasm (22, 28, 32, 33, 64). In the present study, ubiquitin immunostaining clearly showed such inclusions in the remaining lower motor neurons (Figure 4B).

The ultrastructural profiles of Bunina bodies (Figure 5A) and skein inclusions (Figure 5B) were consistent with those described previously (13, 32, 39, 40, 46, 53, 60, 64, 70). We also confirmed this in 5 other cases by reviewing available electron micrographs showing Bunina bodies and skein inclusions.

Ubiquitin-immunoreactive inclusions in cerebral small neurons. Ubiquitinated inclusions are also found occasionally in cerebral non-motor neurons such as neostriatal small neurons (24, 25, 79) and dentate granules cells (2, 51, 80) in ALS with or without dementia. A total of 100 cases were available for this study: 2 cases that showed severe and extensive ischemic changes in the basal ganglia and medial temporal lobe were excluded from the analysis. The results are shown in Table 4.

Ubiquitin-immunoreactive cytoplasmic inclusions were observed in the small neurons of the neostriatum (caudate nucleus and/or putamen). These inclusions were usually small in number and exhibited a crescent or circular profile around the nucleus (Figure 4C). Regardless of the presence or absence of the ubiquitinated inclusions, the neostriatum was unremarkable, without obvious neuronal loss or astrocytic gliosis in all cases. There was no significant difference in the age at onset of ALS, or in the disease duration between cases with the neostriatal inclusions and those without them.

Ubiquitin-immunoreactive cytoplasmic inclusions were also observed in the temporal lobe (dentate granular cells and/or entorhinal small neurons). These inclusions were often round in shape in the dentate granule cells in particular (Figure 4D). Of the 16 cases, 14 also showed the above-mentioned ubiqutinated inclusions in the neostriatum. There was no significant difference in the age at onset, or in the disease duration between cases of ALS with the temporal inclusions and those without them.

Immunoelectron microscopy revealed that the neostriatal ubiquitinated inclusions consisted of coarse granulo-filamentous structures (Figure 5C), the ultrastructural profile being indistinguishable from that of the

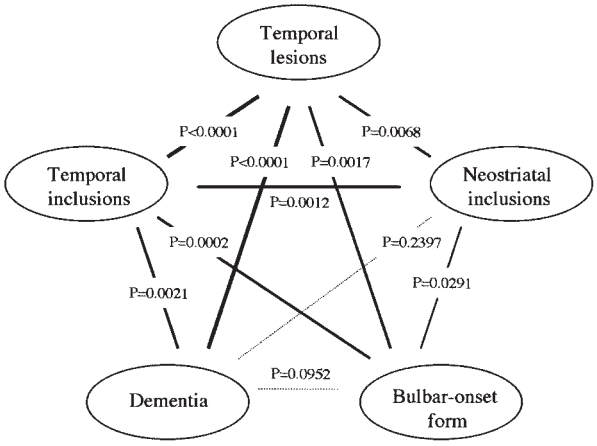

Figure 6. Statistical analysis of the clinical and pathological features among 102 clinicopathologically confirmed cases of sporadic ALS. Solid lines indicate statistically significant associations between the two ($p<0.05$ by chi-squared test).

temporal ubiquitinated inclusions described previously (42, 51).

Similar ubiquitin-positive, tau-negative inclusions may be found in the fronto-temporal cortex in patients with frontotemporal dementia, for which the new classification name of frontotemopral lobar degeneration (FTLD) with motor neuron disease (MND), or FTLD with MND-type inclusions but without MND has recently been proposed (37). Moreover, it has been shown more recently that pathological involvement of the upper and lower motor neuron systems can occur even in the latter (14). In these diseases, fronto-temporal cortical ubiquitin-positive, tau-negative dystrophic neurites have also been described as a characteristic abnormality (14, 16, 20, 58, 71). In the present study, a small number of such dystrophic neurites were found in the neostriatum in 3 (bulbar-onset form/limb-onset form = 2/1) of the 50 cases with neostriatal inclusions (Figure 4E), and in the temporal cortex in 4 (bulbar-onset form/limb-onset form = 3/1) of the 16 cases with temporal inclusions (Figure 4F) (Table 4).

Statistical correlations. From the clinical (dementia, or bulbar- or limb-onset form) and pathological (typical or lower-motor-predominant type, or Bunina bodies, temporal lesions, neostriatal inclusions or temporal inclusions) viewpoints, we performed a further statistical analysis of these ALS autopsy cases. The results are shown in Figure 6. When also analyzing neostriatal and temporal neurites, there were significant correlations between neostriatal neurites and temporal neurites, between neostriatal inclusions and neostriatal neurites,

between temporal inclusions and temporal neurites, between temporal lesions and temporal neurites, and between temporal lesions and neostriatal neurites (p<0.0001each). There were no correlations between these neurites themselves and clinical factors (such as dementia and bulbar-onset form).

Discussion

From 1962 through 2000, 102 autopsy cases of sporadic ALS were seen at our department: the temporal trends in the frequency of occurrence and the age at onset may reflect directly the facts that the incidence of ALS is rising and its rate of occurrence is increasing with age (36, 47-49).

The relationship between mechanical trauma and ALS has so far been studied by many investigators; however, mechanical trauma as a risk factor in the disease is controversial (26, 57). In the present study, we confirmed spondylotic myelopathy in a small number of the ALS cases. This may be of clinical importance, since cervical spondylotic myelopathy in particular is one of the main factors to consider in the diagnosis of ALS (7, 59). We examined the spinal cords from 10 cases of cervical spondylotic myelopathy, including the cases reported previously (17): no Bunina bodies, or ubiquitin-immunoreactive skein or spherical inclusions were observed (data not shown).

In the present study, we observed no expansion of degenerative lesion distribution in cases belonging to the with-ARS subgroup (74). Most (80.4%) of the 102 cases were pathologically definite ALS in which no remarkable histological changes beyond the motor neuron system were observed: of these, 72.0% and 28.0% were typical ALS and lower-motor-predominant ALS, respectively. We found no cases of primary lateral sclerosis (27) or pathologically pure spinal progressive muscular atrophy in the autopsy ledger at our department. Brownell et al (1970) reported obvious pyramidal tract degeneration in 28 (77.8%) out of 36 sporadic, definite cases (5). The rate obtained in the present study was somewhat lower than that reported by the latter investigators. The present study further showed that the age at onset tended to be higher in lower-motor-predominant ALS than in typical ALS. The significance was conserved even when 8 of the 18 cases with temporal lesions and 1 of the 2 cases with PNL degeneration, in which myelin pallor of the corticospinal tract was also unremarkable, were included ($p = 0.0022$). This finding suggests that the effects of ALS, if it is a single entity, on the upper motor neuron system are influenced by the patient's age at onset.

It is now certain that ALS is not simply a disease of the motor neuron system: morphometric studies have indicated that even in typical ALS, significant neuronal loss can be detected in regions other than the motor neuron system (eg, Clarke's column [69], the intermediolateral nucleus [68], and the intermediate zone of the spinal cord [55]). Besides such morphometrically detectable lesions, histologically and/or immunohistochemically apparent neurodegenerative lesions may be encountered beyond the motor neuron system (16, 30). At present, the degenerative lesions that affect the particular regions of the temporal lobe, including the medial cortex of the temporal tip, the pes hippocampi and the amygdaloid nucleus receive much attention in the pathology of ALS (41, 42, 45). The temporal lesions that lack Pick or Alzheimer tau pathology have been discussed in relation to a certain clinical type of ALS: ALS with dementia (15, 38, 75). Nakano (41) showed that such temporal lesions were found in 10 (100%) of 10 ALS cases with dementia and 5 (11.4%) of 44 ALS cases without dementia, and stated that these regions may be the first non-motor cerebral cortex structures to be involved in ALS with dementia. In the present study, we found similar temporal lesions in 6 (85.7%) of 7 ALS cases with dementia and 12 (12.6%) of 95 ALS cases without dementia. Moreover, it was shown that the age at onset tended to be higher and the disease duration tended to be shorter in ALS with temporal lesions than in typical ALS, suggesting that in the former, aging is a risk factor and the prognosis is poorer, often with dementia. It was also of importance that degeneration of the upper motor neuron system was often unremarkable in ALS with temporal lesions; this has also been pointed out in ALS with dementia (38).

It is noteworthy that although rare, PNL degeneration, with (4, 8, 9, 23) or without associated extrapyramidal symptoms (11), has been described in association with ALS: the occurrence of Bunina bodies and ubiquitin-immunoreactive skein inclusions (discussed below) was reported in the remaining lower motor neurons in one case (4). In the present study, we found similar PNL degeneration in 2 cases where the occurrence of Bunina bodies and ubiquitin-immunoreactive skein inclusions was confirmed in the remaining lower motor neurons. However, no signs of basal ganglia disturbance were evident in neither case. We considered the possibility that in these two cases, certain genetic and environmental factors may have been associated with the development of this type of ALS.

In the present study, Bunina bodies were observed in 88 (86.3%) of 102 ALS cases. In previous studies of ALS

(some included a number of familial cases), the following rates were reported: 55 (67.0%) of 82, 20 (90.9%) of 22, 19 (67.9%) of 28, 22 (95.7%) of 23, 20 (64.5%) of 31, and 38 (88.4%) of 43 (Chou, 1979 [6]; Okamoto et al, 1982 [52]; Nakano et al, 1983 [43]; Murayama et al, 1990 [40]; Leigh et al, 1991 [29] and Bergmann, 1993 [3], respectively). The present results again indicated that they are a characteristic cytoplasmic inclusion body in the lower motor neurons in ALS, and also showed that Bunina bodies tend to be found in cases with a shorter disease duration than in those with a longer disease duration. The nature and origin of Bunina bodies remain unclear, although an association between rough endoplasmic reticulum and these bodies has been suggested (65, 66, 70, 73). Bunina bodies are not immunostained with antibodies against Golgi apparatus (35, 63).

Conversely, ubiquitinated skein and/or spherical inclusions were found in the lower motor neurons in 102 (100%) of 102 ALS cases, including cases with temporal lesions and those with PNL degeneration. In previous studies of ALS (again, some included a number of familial cases), the following rates were reported: 23 (100%) of 23, 31 (100%) of 31, 16 (80%) of 20, and 42 (97.7%) of 43 (Murayama et al, 1990 [40]; Leigh et al, 1991 [29]; Schiffer et al, 1991 [62]; and Bergmann, 1993 [3], respectively). The present result further strengthened its importance and significance in the pathology of ALS (33). As in the case of Bunina bodies, the nature and origin of the ubiquitinated inclusions remain unclear. The target protein (or proteins) of ubiquitin for proteolysis in the inclusions remains to be established.

In the present study, we confirmed the occurrence of ubiquitinated cytoplasmic inclusions in the neostriatal and temporal small neurons in ALS with or without dementia, and further showed statistically that bulbar-onset form, temporal lesions, temporal inclusions and neostriatal inclusions are associated with one another, and that as expected, dementia, temporal lesions and temporal inclusions are associated with one another. In addition, ubiquitinated neostriatal and temporal dystrophic neurites, which were first studied systematically in a large number of ALS cases in this study, were found to be associated with dementia and bulbar-onset form through temporal lesions and temporal inclusions. These results strongly supported the hypothesis that ALS and FTLD with MND, and ALS and FTLD with MND-type inclusions but without MND are pathologically closely linked.

In conclusion, the present analytical data, together with the recent temporal trends in the incidence and the age at onset (36, 47-49), suggest that both the clinical and pathological aspects of ALS could change gradually: in the future, ALS may be a more prevalent neurological disorder that is characterized by a higher age at onset, poorer prognosis, bulbar- and lower-motor-predominant abnormality, and often dementia (perhaps frontotemporal dementia [37]). Finally, thus far we have not observed Bunina bodies in non-ALS lower motor neurons. The ubiquitinated inclusions are never immunostained with antibodies against tau, α-synuclein or polyglutamine stretches (10, 34, 72, 76, 79). Early identifications of the proteins that constitute these inclusion bodies would be desirable if we are to elucidate the molecular pathology, and thus the pathomechanisms underlying ALS.

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