Table 2 Case reports of genetically confirmed HD and ALS

Case or references	Age, gender	Family history	CAG repeats	Illness sequence (age at onset)	Initial symptoms of HD	ALS type [16]
Rubio [31]	81[a], M	ALS (mother) Neuropsychiatric disorder (mat. uncle)	45	HD (57) only. Pathologically diagnosed with ALS	Personality change	NA
Papageorgiou [25]	73[b], F	Involuntary movements (brother)	40	HD (72) followed by ALS 18 months later	Involuntary head and limb movements	Limb-onset
Kanai [14]	48[b], M	Involuntary movements (father, siblings)	46	ALS (41) followed by HD 1 year later	Involuntary limb and trunk movements	Limb-onset
Phukan [27]	56[b], M	HD (pat. first cousin)	≥40	ALS (56) followed by HD 7 months later	Involuntary face and limb movements	Limb-onset
Sadeghian [32]	69[b], M	None	40	HD (67) followed by ALS (69)	Memory problems	Limb-onset
Case 1	61[a], F	Chorea (pat. grandmother)	46	HD (early 50's) followed by ALS (59)	Involuntary facial and limb movements	Limb-onset
Case 2	58[a], F	HD (father, pat. grandfather and relatives, sister)	47	HD (mid-30's). Pathologically diagnosed with ALS	Involuntary face, neck and limb movements, Cognitive impairment	NA
Case 3	67[b], F	HD (numerous mat. relatives)	42	HD (50's) followed by ALS (66)	Involuntary limb and trunk movements	Bulbar-onset
Case 4	58[b], F	HD (father)	39	ALS (56) only. Confirmed HD mutation carrier	NA	Bulbar-onset

HD Huntington's disease, *ALS* amyotrophic lateral sclerosis, *NA* non-applicable

[a] Age at death

[b] Supposed age at when the case was reported

motor neurons to TDP-43 associated pathomechanisms and modify TDP-43 pathologic features.

Five patients with genetically confirmed HD who developed ALS-like features have previously been reported [14, 25, 27, 31, 32]. A review of the clinical features in these patients and our patients (Table 2) suggests several common features. First, although the sequence and interval between the onset of HD and ALS varied, the CAG repeat expansions in the HD gene were relatively short in all patients, typically resulting in mid- to later-life symptom onset. Consistent with this, HD onset occurred after at least the mid-30's in all cases, and one patient had not yet developed overt symptoms of HD when ALS symptoms began at age 56. Second, the onset of ALS occurred after the mid-50's in all but one patient. These observations suggest that age-dependent changes may promote the deleterious effect of mutant huntingtin on motor neurons. Similarly, in Machado-Joseph disease, affected individuals who are older and have the smallest disease-causing expansions are most prone to develop motor neuron degeneration [26].

In summary, we suggest the possibility that a rare subset of older HD patients is prone to develop features of ALS with an atypical TDP-43 distribution that in many respects resembles that of aggregated mutant huntingtin. Age-dependent neuronal dysfunction induced by mutant polyglutamine protein expression rarely may contribute to TDP-43 associated pathomechanisms, with a small proportion of the patients developing the phenotypic appearance of motor neuron disease in later life.

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Comparison of phosphorylated TDP-43-positive inclusions in oculomotor neurons in patients with non-ALS and ALS disorders

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ABSTRACT

TDP-43 has been identified as a major component of the pathological inclusions in most forms of frontotemporal lobar degeneration with ubiquitin-positive inclusions and in amyotrophic lateral sclerosis (ALS). In the present study, paraffin sections of the midbrain in 112 patients with various non-ALS disorders and 27 patients with sporadic ALS were immunostained with antibody against phosphorylated TDP-43 (pTDP-43). pTDP-43-positive inclusions in oculomotor neurons were detected in 18 of 112 patients with non-ALS disorders (16.1%). The appearance of the inclusions showed fine filamentous structures rather than the skein-like inclusions seen in the anterior horn cells of ALS spinal cords. The incidence was increased in the age range of 80–89 years old (10/37 cases; 27.0%), in which 6 of 10 cases demonstrated AD pathology in the temporal lobes. Twenty-seven ALS patients were examined and the findings were compared with those of non-ALS patients. There were 13 cases demonstrating pTDP-43-positive inclusions (48.1%) which showed stronger immunoreactivities in ALS cases. This is the first report demonstrating fine filamentous pTDP-43-positive inclusions in oculomotor neurons in non-ALS disorders. Although the mechanisms underlying pTDP-43 in oculomotor neurons are currently unknown, its detection is of interest, and the expression may occur not only in ALS but also during the aging process.

1. Introduction

TAR-DNA-binding protein 43 kDa (TDP-43) is a nuclear protein that functions in regulating transcription and alternative splicing of mRNA [1,2]. In 2006, it was found that TDP-43 is a major component of inclusions in frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U) and in amyotrophic lateral sclerosis (ALS) [3,4], and these two diseases are now referred to as FTLD-TDP or ALS-TDP, respectively [5,6]. ALS is now recognized as a multisystem degenerative disease widely affecting neurons and glia with a heterogeneous pattern of TDP-43 pathology [7]. Recent detailed clinico-pathological studies showed that the accumulation of TDP-43 is detected in many diseases including Alzheimer disease (AD) [8–13], dementia with Lewy bodies [12,14], Pick's disease [12,15], hippocampal sclerosis [8], corticobasal degeneration [13], Huntington's disease [16], and argyrophilic grain disease [17] with secondary TDP-43 pathology. Such TDP-43 pathology is predominantly observed in the medial temporal lobes, however, the pathological significance of TDP-43 remains unclear. A few studies are reported looking at changes in TDP-43 associated with aging, in which TDP-43 inclusions are found in approximately 3% of normal individuals and restricted to the hippocampus and entorhinal cortex [14,18].

Phosphorylation-independent and phosphorylation-dependent antibodies to TDP-43 demonstrated that normal TDP-43 is physiologically localized within normal nuclei, while TDP-43 is phosphorylated in the pathological inclusions [19–21]. Furthermore, phosphorylation promotes the oligomerization and fibrillization of TDP-43 in vitro [19]. Abnormal phosphorylation of TDP-43 takes place on at least five sites near the carboxy-terminal region [19], which occur similarly in tauopathies [20] and α-synucleinopathies [22]. These findings suggest that abnormal phosphorylation of TDP-43 should be a critical step in the pathogenesis of ALS. We have recently generated an antibody recognizing phosphorylated TDP-43 (pTDP-43) at position 409 and 410 [23]. Our antibody detects a single band at about 45 kDa and smaller fragments at about 25 kDa with smears in ALS and AD samples, and demonstrates dot-like inclusions in the brains of AD patients and aged subjects [23].

When we examined the brainstem and hippocampus to determine the expression of pTDP-43 in a series of autopsied cases using anti-pTDP-43 antibody, we unexpectedly found fine filamentous pTDP-43-positive inclusions in the oculomotor neurons of patients with non-ALS disorders. We considered these inclusions similar to skein-like inclusions. In this study, we evaluated the characteristics of these filamentous inclusions in non-ALS disorders, and compared these structures with the skein-like inclusion of ALS patients.

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2. Materials and methods

We examined mainly the midbrain in a total of 112 patients diagnosed with various kinds of non-ALS diseases (age range: 17–97 years old, average age: 74.0 years old, 72 males, 40 females) and 27 patients with sporadic ALS (age range: 44–79 years old, average age: 63.0 years old, 14 males, 13 females). Autopsied samples were all obtained from our laboratory and the Geriatric Research Hospital. In all cases, the autopsies were performed in accordance with established procedures and the samples were used in this study after obtaining informed consent from the family of each patient. ALS patients were definitively diagnosed based on clinical and light microscopic findings. Samples were fixed with 4% paraformaldehyde in phosphate-buffered solution (PBS) (pH 7.4) and embedded in paraffin. Five-μm-thick transverse paraffin sections were prepared for Hematoxylin-Eosin (H&E) staining and then immunohistochemistry, which was carried out using a rabbit polyclonal anti-phosphorylation-dependent TDP-43 (pTDP-43) antibody (1:3000) generated in our laboratory [23]. For enhancement, the samples were autoclaved for 5 min before reaction with the antibody. Sections were blocked in normal horse serum for 30 min at room temperature, then labeled with the first antibody at 4 °C overnight, washed in PBS for 30 min, incubated with the second antibody provided in the Histofine SAB-PO kit (Nichirei, Tokyo, Japan), washed in PBS for 30 min, and finally visualized by the avidin-biotin-peroxidase method. Observation was performed using an Olympus BX50 microscope.

To further confirm whether our antibody recognized the characteristic structures in ALS, we immunostained three-μm-thick mirror sections of ALS spinal cords using anti-pTDP-43 antibody and either a rabbit polyclonal anti-phosphorylation-independent TDP-43 (TDP-43) antibody (1:3000, 10782-1-AP; Proteintech Group, Chicago, IL) or a rabbit polyclonal anti-phospho TDP-43 antibody (1:3000, pS409/410-1; Cosmo Bio, Tokyo, Japan), and compared the immunoreactivities of our antibody with those of the other two commercial antibodies. Autoclaved treatment was performed for both antibodies.

We also immunostained temporal lobes including the hippocampus and midbrain using a mouse monoclonal anti-phosphorylated tau protein (1:200, AT8; Innogenetics, Ghent, Belgium) and a mouse monoclonal anti-amyloid β protein (1:5000, 4 G8; Covance, Emeryville, CA) antibodies in addition to anti-pTDP-43 antibody. Autoclaved treatment was performed for AT8 and pretreatment of tissue using formic acid (70%) for 10 min was performed for 4 G8.

3. Results

Firstly, we immunostained the mirror sections of ALS spinal cords using our generated anti-pTDP-43 antibody and anti-TDP-43, one of the commercially available antibodies, and then compared both immunoreactivities to determine whether the anti-pTDP-43 antibody recognized the characteristic structures of ALS similarly to anti-TDP-43 antibody. The results demonstrated that pathological structures including skein-like inclusions, round inclusions, and dot-like inclusions were similarly immunostained in the anterior horn cells by both antibodies to pTDP-43 (Fig. 1A) and TDP-43 (Fig. 1B). Differing from the anti-TDP-43 antibody that recognizes normal nuclei of the cells, anti-pTDP-43 antibody did not immunostain the nuclei regardless of whether neuronal cytoplasmic inclusions (NCIs) were present. Both antibodies detected NCIs (Fig. 1A and B) and glial cytoplasmic inclusions in a similar manner (arrowhead in Fig. 1A and B). We also compared the immunoreactivities of ALS spinal cord between anti-pTDP-43 antibody (Fig. 1C) and anti-pS409/410-1 antibody (Fig. 1D), the other commercially available antibody. ALS-characteristic structures in neuron and glia were similarly recognized with both antibodies. These results suggested that anti-pTDP-43 antibody was useful

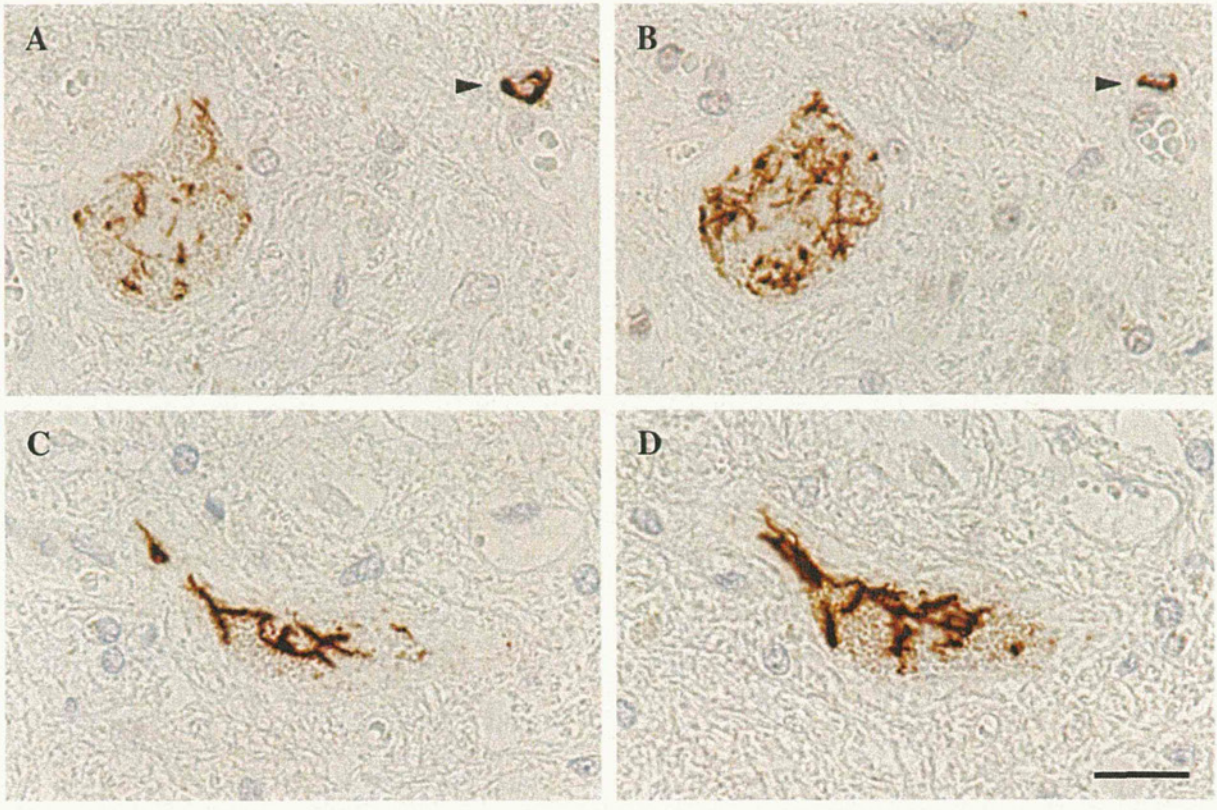
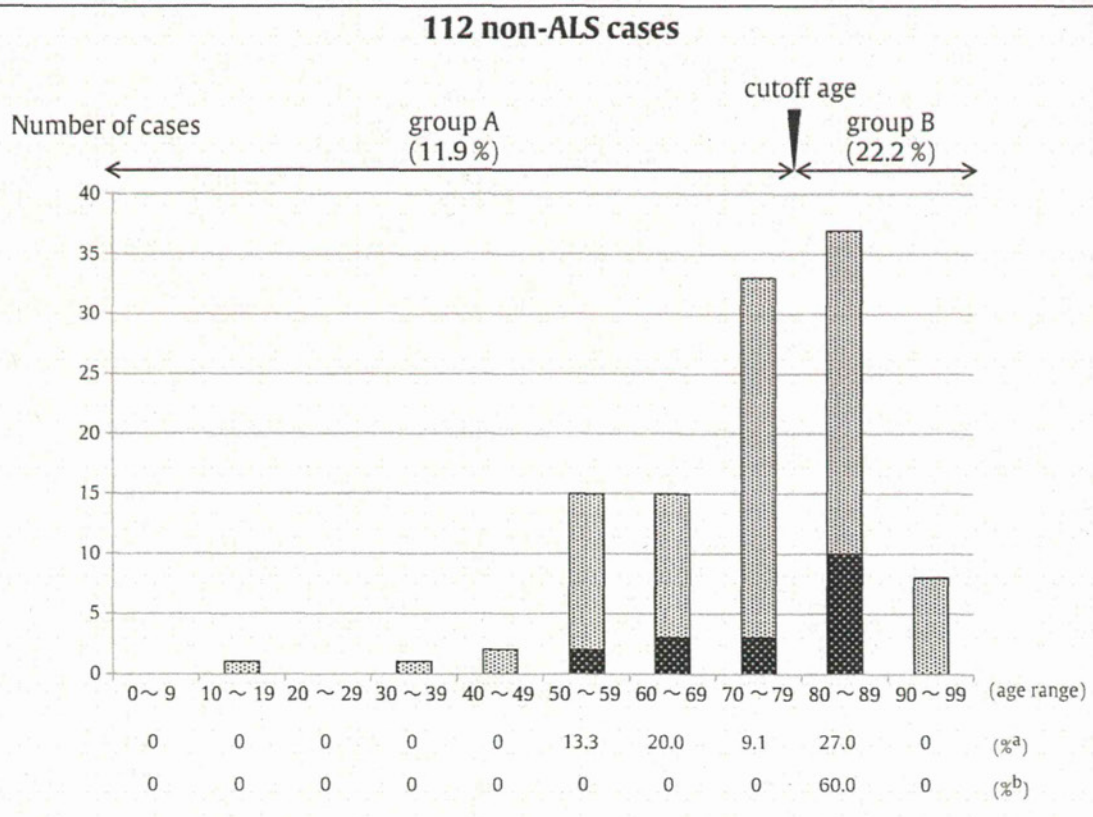


Fig. 1. pTDP-43, TDP-43, and pS409/410-1 immunoreactivities of anterior horn cells of spinal cords from patients with ALS. A, C: immunostaining of anti-pTDP-43 antibody. B: immunostaining of anti-TDP-43 antibody. D: immunostaining of anti-pS409/410-1 antibody. Immunostainings for skein-like inclusions with anti-pTDP-43 antibody (A and C) were similar to those with anti-TDP-43 antibody (B) and anti-pS409/410-1 antibody (D). Arrowheads indicate glial cytoplasmic inclusions (A and B). Scale bar: 20 μm.

to detect the pathological structures as well as antibodies to TDP-43 and pS409/410-1.

Secondly, we examined the midbrain of 112 non-ALS cases, focusing on oculomotor nuclei to determine if there were some structures immunostained with anti-pTDP-43 antibody. Eighteen of 112 cases showed neuronal cytoplasmic pTDP-43-positive immunoreactivities in oculomotor neuron (16.1%). The number of these neurons with inclusions ranged from 1 to 8 in each section. The final pathological diagnoses of 18 non-ALS disorders (age range: 59–85 years old, average age: 75.8 years old, 14 males, 4 females) were various, and no symptoms suggestive of ALS-like phenomena were observed. The details were as follows; three cases of acute myocardial infarction, two cases of cerebral infarction, four cases of cerebral bleeding, each one case of subarachnoid hemorrhage, cerebral amyloid angiopathy, and lung abscess, and six cases of malignant tumor such as two cases of lung cancer, one case of malignant lymphoma, one case of renal, adrenal, and pancreatic cancers, one case of gastric cancer, and one case of soft tissue sarcoma of the abdomen. The duration of the illness from onset was not very long, ranging from 1 day to 11 months. The number of cases with and without inclusions in each age group is demonstrated in Table 1. Autopsied cases clustered in the age group of 50 years and older, and the frequency of cases with inclusions was especially increased at 80–89 years old. When these 112 cases were divided into two age groups with a cutoff age of 80 years old, 67 and 45 patients belonged to groups A and B, respectively, in which 8 cases in the former group and 10 cases in the latter group showed pTDP-43-positive inclusions. In this series, the frequencies of the presence of inclusions of groups A and B were 11.9% (8/67 cases) and 22.2% (10/45 cases), respectively. The percentage of cases with inclusions to total cases of each age group was high in the group aged 80–89 years old (10/37 cases; 27.0%) and roughly increased as age was growing up. The most prominent appearance of pTDP-43-positive structures demonstrated single fine filamentous inclusions (Fig. 2A and B), which were located in the cytoplasm. In addition, there were other inclusions composed of complicated filaments (Fig. 2C), although these inclusions seemed faint when compared to skein-like inclusions of anterior horn cells of ALS spinal cords (See Fig. 1). Other than the filamentous structures, dot-like inclusions were also observed, which might have been caused by diagonal cutting of filamentous inclusions (Fig. 2D). Using the anti-TDP-43 and anti-pS409/410-1 antibodies, we could not detect any similar structures in oculomotor neurons (data not shown). In addition, there were neither pTDP-43-positive cytoplasmic inclusions in the brainstem including the hypoglossal nucleus, nor pTDP-43-positive glial

Table 1
112 non-ALS cases. The number of the cases demonstrating pTDP-43-positive inclusions was highest in the group aged 80–89 years old (10 cases). The number of cases with inclusions to total cases of each age group is shown in percentage, and highest in the group aged 80–89 years old (27.0%). AD pathology was confirmed in 6 of 18 cases (33.3%), all of which cases were in the group aged 80–89 years old (60.0%; 6/10 cases).



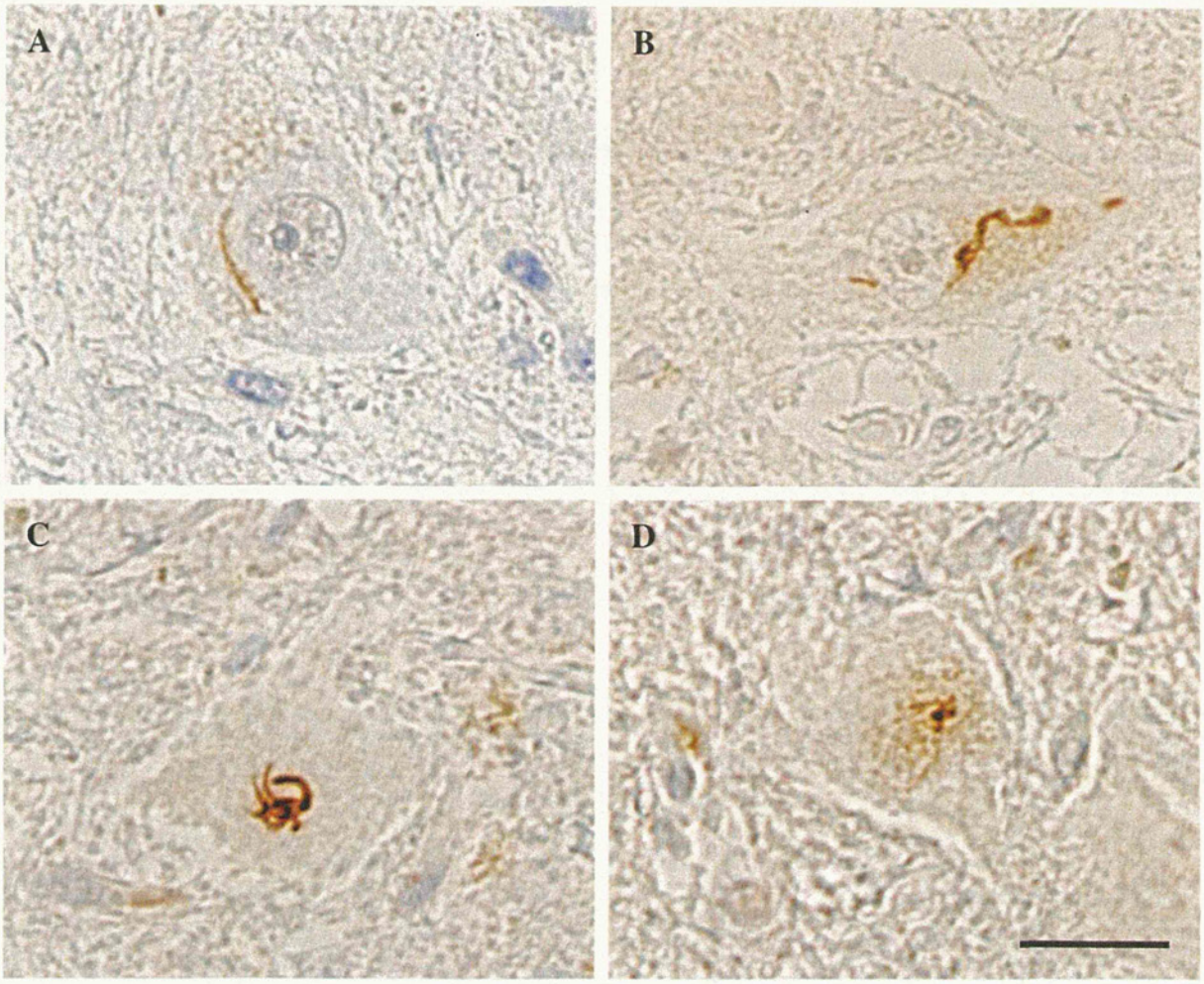


Fig. 2. pTDP-43 immunoreactivities of oculomotor neurons in patients with non-ALS disorders. A, B: a piece of fine filamentous inclusions. C: complicated fine filamentous inclusions. D: dot-like inclusions. The pTDP-43-positive inclusions of non-ALS cases were simpler than those in A and C in Fig. 1 and A in Fig. 3. Scale bar: 20 μm.

inclusions in the midbrain including the substantia nigra nor any in the brainstem or temporal lobe in these 18 cases (data not shown). pTDP-43-positive granulovacuolar degenerations were frequently observed in the hippocampus of elderly subjects (data not shown), however, pTDP-43-positive fine filamentous inclusions were not observed in the temporal lobes.

Thirdly, we examined the midbrain of a total of 27 sporadic ALS patients, focusing on the oculomotor nuclei to determine whether pTDP-43-positive structures were similarly present. Although oculomotor nuclei were apparently well preserved with H&E staining, the antibody to pTDP-43 occasionally demonstrated NCIs in 13 of 27 cases (48.1%). These inclusions (Fig. 3A) appeared to be the same as the skein-like inclusions in anterior horn cells of ALS spinal cords. In addition, 5 of 27 cases demonstrated a more marked presence of round inclusions in oculomotor neuron (Fig. 3B), and 3 of these cases also showed dot-like inclusions (Fig. 3C). A total of 7 cases containing either round, dot-like, or both inclusions commonly showed inclusions in oculomotor neuron (25.9%; 7/27 cases), and all of these cases showed pTDP-43-positive glial inclusions in the area of oculomotor neurons and central gray matter (Fig. 3D). Furthermore, the frequencies of pTDP-43-positive NCIs in the neurons in the substantia nigra and hippocampal granular cell layer were 63.0% (17/27 cases) and 37.0% (10/27 cases), respectively, in which 10 cases in the former (58.8%) and 7 cases in the latter (70.0%) indicated pTDP-43-positive inclusions in oculomotor neuron. Finally, the number of cases demonstrating wider distribution of pTDP-43-positive inclusions including central gray matter, substantia nigra, and the hippocampal granular cell layer was 5, and all the cases showed pTDP-43-positive inclusions in the oculomotor neuron. Using the anti-TDP-43 and anti-pS409/410-1 antibodies, the skein-like inclusions were also detected in oculomotor neurons (data not shown). However, Bunina bodies were not observed in oculomotor neurons in all cases examined.

To determine if the 18 non-ALS cases were related to AD pathology, we performed additional experiments using antibodies of AT8 and 4 G8. Detailed pathological examination showed a large number of neurofibrillary tangles (NFTs) and senile plaques (SPs) in the temporal lobes including the hippocampus, which were compatible to AD in 6 cases in the age range of 80–89 years old (Table 1). The percentages of cases with inclusions showing AD pathology to total cases with inclusions and to the age group of 80–89 years old were 33.3% (6/18 cases) and 60.0% (6/10 cases), respectively. In addition, SPs were seen in 3 of 6 cases around the central gray matter of the midbrain. On the other hand, the remaining 12 non-ALS cases showed normal aging pathology.

4. Discussion

While we screened the serially autopsied brains using anti-pTDP-43 antibody, we unexpectedly found a fine filamentous pTDP-43-positive inclusion in oculomotor neurons in 18 of 112 patients with non-ALS disorders. Based on the appearance and immunoreactivities

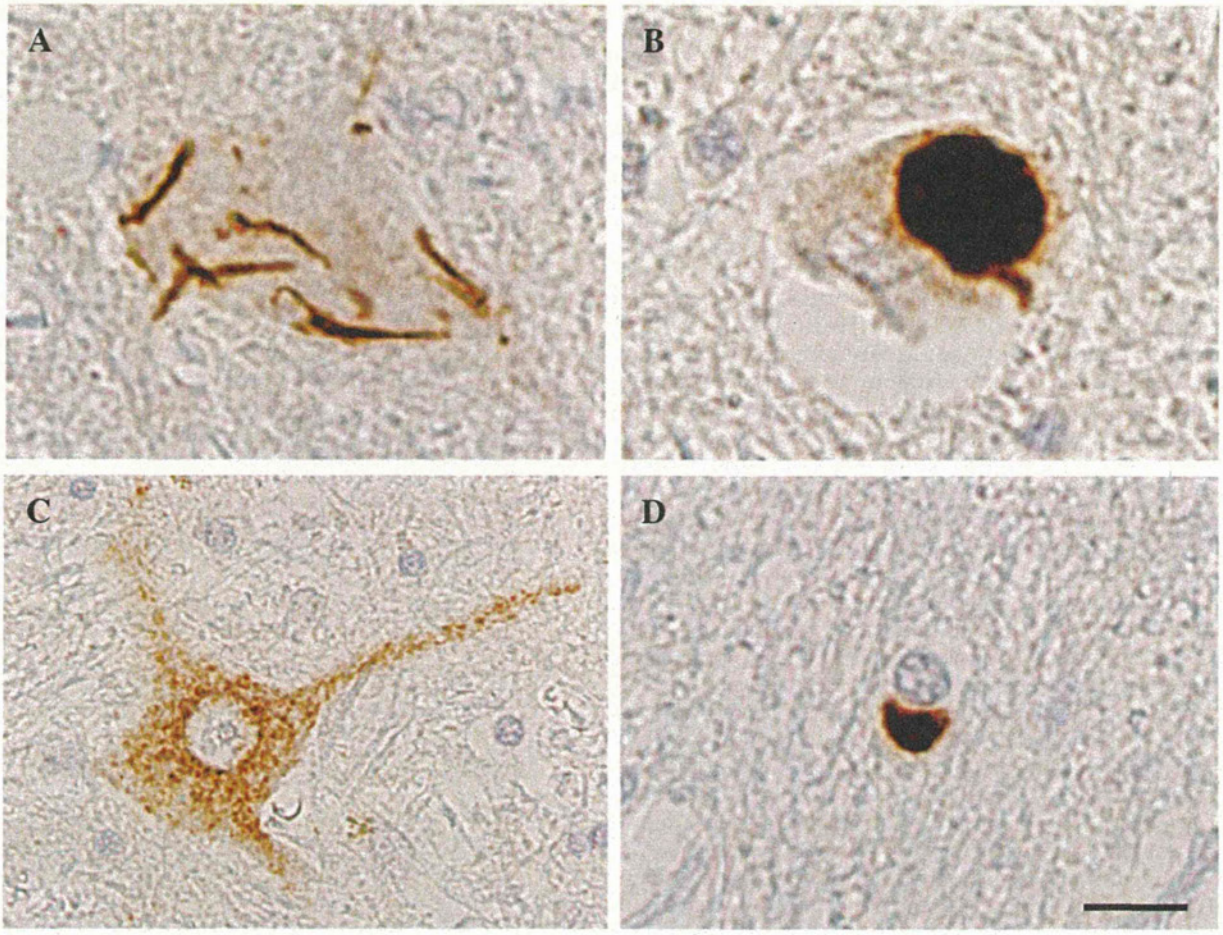


Fig. 3. pTDP-43 immunoreactivities of oculomotor neurons in patients with ALS. A: skein-like inclusions. B: round inclusions. C: dot-like inclusions. D: glial cytoplasmic inclusions. Skein-like inclusions of ALS cases showed much stronger immunoreactivities than those in Fig. 2. Scale bar: 20 μm.

with anti-pTDP-43 antibody, we suspected that these structures were similar to skein-like inclusions seen in ALS. The question arises as to whether a piece of fine filamentous inclusion detected in non-ALS disorders was derived from the same origin as skein-like inclusions in ALS patients.

In this study, we evaluated the appearance, presence, and number of pTDP-43-positive inclusions, and compared these findings between non-ALS and ALS cases. Comparison of the appearance in non-ALS with that in ALS suggested that the two types of inclusions were somehow dissimilar. When we immunostained the midbrain of non-ALS disorders, using commercially available polyclonal antibodies to phosphorylation-independent TDP-43 and phosphorylation-dependent TDP-43 (pS409/410-1), we could not detect the fine filamentous structures in non-ALS samples. The first possibility is that as the number of inclusions was extremely limited, it was rather difficult to detect them. The difference in the synthetic peptides as antigen could be the second possibility. Our peptides were one amino acid longer, corresponding to amino acid residues #404-414 in human TDP-43, but only #405-414 for anti-pS409/410-1 antibody [19], where non-phosphorylated serine was added to the synthetic peptides (#405-414) at position 404. It is unknown what effects the serine has on the results of immunohistochemical analysis. The simple difference in a peptide of non-phosphorylated serine could be the key to resolve the immunoreactive diversity. The third possibility could be the diversity of components comprising the inclusions. Although pTDP-43 was commonly identified in the inclusions of both non-ALS and ALS cases, whether the rest of the components would be the same is unknown. The possibility that the components would not be the same might be supported by the findings that anti-TDP-43 or anti-pS409/410-1 antibodies did not detect the inclusions in non-ALS cases but in ALS cases. The fourth possibility is that our antibody, different from the other commercial antibodies, would be more advantageous for detecting the abnormal proteins in the inclusions. Further examinations including immunohistochemical and electron microscopic analyses could offer a hint toward determining whether both inclusions are composed of the same proteins.

There arises another query as to why the inclusions in non-ALS disorders showed very fine filamentous immunoreactivities. The possibility is that whether these inclusions were either simpler or more complicated would depend on the time when the formation of inclusions started. In the earlier stage, immunoreactive structures could indicate fine filamentous inclusions. As time passes, the inclusions would mature and become more complicated than the simpler structures. If further disease-related pathological degeneration affected the brain, immunoreactivites would be much brighter. If this hypothesis were true, it would explain why the immunoreactivites of inclusions were stronger in ALS samples, and the stronger immunoreactivites of inclusions could reflect the disease-related degeneration. In this study, skein-like, round, or dot-like inclusions were seen in the oculomotor neurons of 13 of 27 ALS cases (48.1%), and the frequency of cases demonstrating inclusions was similar to the previous findings reported by Nishihira et al. (13 of 31 cases; 41.9%) [7]. Our findings showed a tendency for pTDP-43-positive inclusions in oculomotor neurons to be increased in ALS cases when a wider distribution of pTDP-43-positive inclusions in the area of central gray matter, substantia nigra, and hippocampal granular cell layer, corresponding to the type-2 distribution pattern [7], was observed.

Whether the presence of oculomotor neurons containing fine filamentous inclusions was detected partly depended on aging in non-ALS cases. If the age cutoff was set at 69 years old and under, the frequency of the presence of inclusions in oculomotor neuron was 14.7% (5/34 cases) in group A and 16.7% (13/78 cases) in group B, indicating no significant difference between the two groups. When the establishment of the age cutoff was set at 79 years old and under, the frequency was increased 1.86 fold in group B (22.2%; 10/45 cases) compared to group A (11.9%; 8/67 cases). Among 18 non-ALS cases showing pTDP-43-positive inclusions, 6 cases in the age range of 80–89 years old could be compatible to AD pathology because of a widespread distribution of NFTs and SPs in the temporal lobes including the hippocampus [24–26]. As such, we speculate that the aging processes could play some role in forming these inclusions. In other words, longevity would be partly involved in the occurrence of the inclusions in non-ALS disorders, because the central nervous system is expected to show some degeneration as people with age. However, findings were contrary to our expectations when 8 patients who passed away at the age of 90 years old and over (age range: 90–97 years old, average age: 92.9 years old) were examined. These cases did not show pTDP-43-positive inclusions in the oculomotor neuron. In addition to the normal aging, there could be several environmental factors such as mental stress, a history of trauma to the brain [27], some types of neurodegeneration, and poisoning that could be associated with the presence of these inclusions. With respect to the duration of illness, oculomotor neurons containing inclusions were observed in 4 non-ALS patients who suddenly died of acute myocardial infarction, cerebral infarction, or subarachnoid hemorrhage within 12 days after these events occurred. These findings suggest that a long interval after onset is not necessary to produce an abnormal accumulation of pTDP-43-positive inclusions.

In conclusion, this is the first report demonstrating fine filamentous pTDP-43-positive inclusions in the oculomotor neurons in non-ALS disorders. Our findings suggest that although the mechanisms underlying pTDP-43 in oculomotor neurons are currently unknown, its detection is of interest, and the expression may occur not only in ALS but also during the aging process.

Conflict of interest

The authors declare that there is no conflict of interest.

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Activation and alteration of lysosomes in multiple system atrophy

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Multiple system atrophy (MSA) is a sporadic neurodegenerative disorder. Its histopathological features include glial cytoplasmic inclusions that contain α-synuclein as the main component. Recently, multiple lines of evidence have suggested a role for lysosomes in the pathogenesis of many neurodegenerative diseases. To elucidate whether lysosomes are also implicated in the pathology of MSA, we carried out an immunohistochemical study using antibodies against lysosomal proteins in the brains of patients with MSA and in control brains. A robust increase in the expression and an alteration in the morphology and distribution of lysosomal-protein-positive structures were observed in MSA brains. Double immunohistochemistry demonstrated that lysosomal markers did not colocalize mainly with glial cytoplasmic inclusions, but colocalized with a microglial marker. These immunohistochemical signatures suggest that lysosomes are activated in microglia during the disease process, and play a pivotal role in the pathology of MSA. *NeuroReport* **23:270–276** © **2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Accumulation and deposition of misfolded proteins are common signs of neurodegenerative diseases. The regional distribution in the brain and the composition of protein aggregates are different in each neurodegenerative disease [1]. For example, deposition of amyloid β protein and phosphorylated tau (pTau) and accumulation of α-synuclein in Lewy bodies are the main pathological features of Alzheimer's disease (AD) and Parkinson's disease (PD), respectively [2,3]. Recently, dysfunction of protein degradation systems was proposed as one of the causes for the accumulation of these aberrant proteins.

A normal balance between the formation and the degradation of cellular proteins is required for cell survival. The ubiquitin–proteasome and the autophagy–lysosome systems are two important cellular systems that are responsible for the degradation of misfolded proteins [4]. The ubiquitin–proteasome system is a protein degradation system that involves the modification of target proteins with ubiquitin, which signals the target protein for degradation by a multisubunit protease, termed proteasome. The autophagy–lysosome system is mainly responsible for the nonselective degradation of proteins. In the processes of the autophagy–lysosome system, double membrane-bound structures called autophagosomes fuse with lysosomes and the contents of the autophagosomes are digested by lysosomal enzymes. Lysosomes are composed of soluble acidic hydrolases, integral membrane proteins, and membrane-associated proteins [5]. Dysfunction in any of these components may cause lysosomal deficits, leading to the accumulation of undegraded metabolites and, ultimately, diseases that cause neurodegeneration. Lysosomal alteration is observed in many neurodegenerative diseases, even if lysosomal disturbance does not represent the direct cause of disease [5,6]. This indicates that lysosomes play a role in neurodegeneration.

Multiple system atrophy (MSA) is a sporadic neurodegenerative disorder that is characterized by neuronal loss accompanied by gliosis in the basal ganglia, cerebellum, pons, inferior olivary nuclei, and the spinal cord. The histopathological features of MSA include the presence of argyrophilic glial cytoplasmic inclusions (GCIs) in oligodendroglia [7], which contain α-synuclein as the main component [8]. Initial reports regarding the clearance of α-synuclein suggested that this protein is degraded by the proteasome [9]. However, it has been reported recently that α-synuclein is also degraded through the autophagy–lysosomal pathway [10,11]. Alterations in proteasomal and lysosomal markers were observed in PD brains [12]. The ubiquitin–proteasome system plays a role in MSA brains, because ubiquitin is one of the major components of GCIs [13]. In contrast, the participation of the autophagy–lysosome pathway in MSA pathogenesis has not been reported. These findings motivated us to study lysosomal alteration in MSA brains. In this study, we used immunohistochemistry to demonstrate the alteration of lysosomal proteins in MSA samples. Lysosomal activation and alteration were observed in MSA brains. These data support the involvement of the autophagy–lysosome pathway in MSA pathogenesis.

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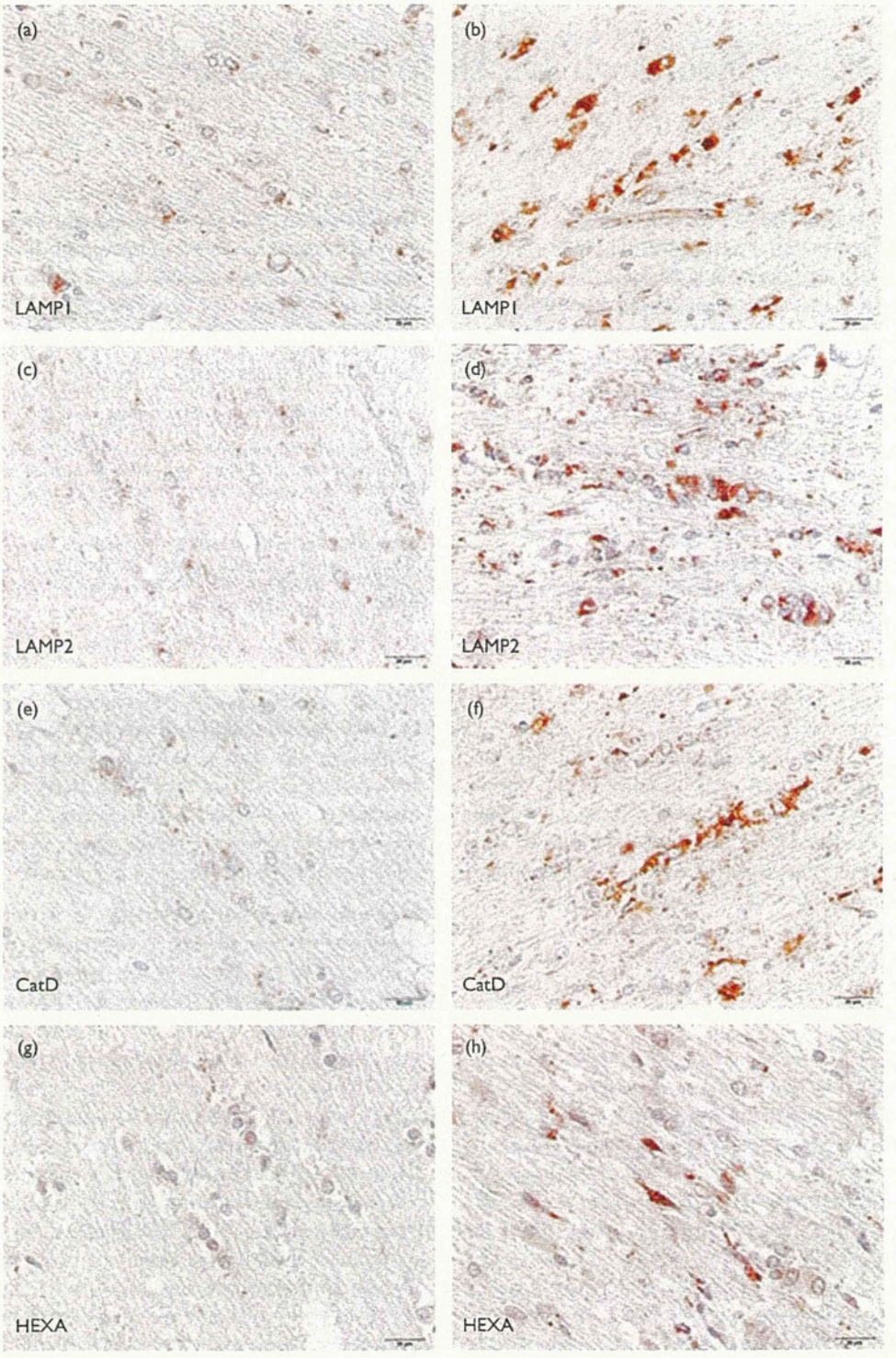
Fig. 1



Immunohistochemical detection of lysosomal proteins (LAMP1, LAMP2, cathepsin D, and HEXA) in control brains (a, c, e, and g) and MSA brains (b, d, f, and h). A robust increase in LAMP1 (b), LAMP2 (d), cathepsin D (f), and HEXA (h) staining was observed in MSA brains. The intensity of the staining in MSA brains was much stronger than that in control brains, and the immunoreactivity area was extensive in the cytoplasm of many cells. HEXA, hexosaminidase subunit A; LAMP, anti-lysosomal-associated membrane proteins; MSA, multiple system atrophy.