based on a nationwide scale sample. In Japan, the proportion of the ALS patients with TPPV is relatively higher than in other countries [10,11]. Rare symptoms such as ophthalmoplegia are more frequently seen in those who receive TPPV to prolong survival [12,13], so the clinical profile of ALS patients in Japan might have unique features. Data concerning the clinical features are important to establish an early diagnosis, treatment plan, and prognostic estimation, as well as to design clinical trials.

The aim of this study was to profile the detailed clinical features of sporadic ALS on large-scale samples in Japan.

2. Research design and methods

A nationwide registration of patients with intractable diseases including ALS has been conducted by the Ministry of Health, Labor and Welfare of Japan since 1974. When a patient is diagnosed as having ALS, the patient can apply for registration in this system, and receive financial support from the state for medical expenses incurred for the treatment of ALS, independent of the disease severity. In 2003, a data collection system was developed for research use of this registration system. Concurrently with that, the registration form for ALS was revised substantially. Since 2003, the annual renewal of registration of each patient has been conducted. The data from registration forms were input to the database in each prefectural office and consolidated in the Ministry of Welfare, Health and Labor of Japan. In the revised registration form, the overview of the clinical state is to be indicated, including the severity, neurological symptoms, activities of daily living and conditions of tube feeding or non-invasive positive pressure ventilation (NIPPV) and TPPV of ALS patients in Japan on a nationwide scale. Using the data accumulated from 2003 to 2006, we analyzed the clinical features of sporadic ALS patients in Japan. Clinical profiles of sporadic ataxias in Japan were previously described using this registration system [14].

The inclusion criteria of the registration system for ALS are: 1) adult onset, steady progressive course; 2) the presence of clinical or electrophysiological evidence of lower motor neuron (LMN) degeneration in at least two topographical anatomic regions (brainstem, cervical, thoracic or lumbosacral region), together with clinical evidence of upper motor neuron (UMN) degeneration in at least one region; and 3) the absence of electrophysiological and pathological evidence of other disease processes that might explain the signs of LMN and/or UMN degeneration, and neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs. Therefore the patients registered in this system satisfy definite, probable or possible ALS based on the revised El Escorial Criteria [15] for the diagnosis of ALS.

The data collection system was developed in 32 of 47 prefectures in Japan. In proportion to the total population, 63% of total registered patients in Japan were integrated into the computerized database. The data were comprised of initial registration form and renewal registration form. When a patient was diagnosed as ALS, the initial registration form was used to apply for the system, and the renewal registration form was used in the following year. However, the information on the patients initially registered before 2003 was comprised of data from only renewal registration.

After 2003, 3694 ALS patients were newly registered in the system. Records were eliminated from the analysis if information was missing for age at onset and age at registration. Ninety-four patients were also excluded who had a family history of motor neuron disease or an abnormality of genes related to neurodegenerative disease such as the SOD1 mutation. The inclusion age range was above 20 years at onset. After these data clearing, the data from a total of 3428 patients were available. In order to analyze the age at onset, initial symptoms and related clinical features, we used this data set.

In a single year, 2005, 4546 ALS patients were registered using the initial registration form or renewal registration form. The number included those initially registered before 2003. To describe the cross-sectional overview of the medical and social conditions of ALS patients in Japan, we used this data set. After the data described above were excluded, the data from 4202 patients were used.

From 2003 to 2006, 2440 ALS patients with TPPV were registered at least once, mostly using the renewal registration form. The number included those initially registered before 2003. To describe the conditions of ALS patients with TPPV, we analyzed this data set. After the data cleaning, the data from 2128 patients with TPPV were used.

All of the patients provided written informed consent for the research use of the data, and the anonymity of the data was strictly secured. We implemented the guidelines for research use of the data from the nationwide registration system of intractable diseases and the ethics guidelines for clinical studies endorsed by the Japanese government. The research project was approved by the Ministry of Health, Labor and Welfare, Japan, and by the ethics committee of Nagoya University Graduate School of Medicine.

2.1. Assessment of clinical features

Age at onset was considered as the time of the patient's initial awareness of weakness. As for the initial symptoms, six symptoms including dysarthria, dysphasia, respiratory disturbance, weakness of neck, weakness of upper extremities and weakness of lower extremities were noted. In most cases, one symptom was assessed as an initial symptom, however, two or more symptoms may be recorded. The activities of daily living and clinical symptoms were assessed by 6 items from the 12 items of ALSFRS-R (Speech, Swallowing, Handwriting, Dressing and Hygiene, Walking and Dyspnea). The Japanese version of ALSFRS-R was validated previously for ALS, showing that the assessment values are highly equivalent among well-trained neurologists, general physicians and nurses, and that intra-rater assessment values are also highly equivalent [16]. Intra-rater and inter-rater reliability of each item of the Japanese version of ALSFRS-R were also validated. The presence of oculomotor disturbance was assessed through a bedside neurological examination.

2.2. Data analysis

All variables were summarized using descriptive statistics, including mean, standard deviation (S.D.), and percentages. Correlations

Table 1
Clinical features of patients newly registered from 2003 to 2006 (n=3428)

Age at onset (years, mean±S.D.)	65.4±10.7
Male/female (%)	57.8/42.2
Duration from disease onset to registration (years, mean±S.D.)	1.7±2.2
Symptoms at registration (%)	
Dysarthria	64.2
Dysphagia	57.8
Weakness of neck	70.0
Respiratory distress	34.2
Weakness of upper extremities	86.6
Weakness of lower extremities	76.2
Initial symptoms (%)	
Dysarthria	36.3
Dysphagia	21.1
Weakness of neck	7.1
Respiratory disturbance	6.3
Weakness of upper extremities	48.1
Proximal dominant	26.1
Distal dominant	50.8
Diffuse	23.0
Weakness of lower extremities	34.1
Proximal dominant	19.7
Distal dominant	42.6
Diffuse	37.8

Table 2
Cross-sectional living conditions of patients registered in 2005 (n=4202)

Living condition	Frequency (%)
At work or school	6.7
Household work	6.5
Under home care	58.2[a]
In hospital	27.5[a]
In nursing-care facility	2.4

[a] 1.2% of patients overlap.

between age at onset and duration from disease onset to invasive procedures were analyzed using Pearson's correlation coefficient, and the cumulative incident curves of two age groups were assessed by the log-rank test. Difference of frequencies of symptoms between two age groups was assessed by the chi-square test. p-values <0.05 were considered to be statistically significant. Calculations were performed using the statistical software package SPSS 15.0J for Windows (SPSS Japan Inc., Tokyo Japan).

3. Results

3.1. Clinical features of sporadic ALS patients

The mean age at onset was 65.4±10.7 years, the male to female ratio was 1.37:1, and the mean duration from disease onset to registration was 1.5±1.4 years. The initial symptom was dysarthria in 36.3%, dysphagia in 21.1%, weakness of neck in 7.1%, respiratory disturbance in 6.3%, weakness of the upper extremities in 48.1%, weakness of lower extremities in 34.1%, when allowing overlapping descriptions (Table 1). When we analyzed these demographic clinical features between male and female patient groups, age at onset was slightly higher in the female patients. The proportion of the patients with bulbar symptom onset was higher in the female patients, whereas, the proportion of the patients with weakness of upper extremities was higher in the male patients (Supplemental Table 1).

The cross-sectional state of living conditions of ALS patients in Japan in 2005 is shown in Table 2. The proportion of the patients at work or school was 6.7%, 6.5% engaged in household work, 58.2% under home care, 27.5% in hospital and 2.4% in a nursing-care facility. The state of nutrition and respiratory support is shown in Table 3. The frequency of patients with a gastrostomy tube was 28.7%, and 7.8% were using a nasogastric tube. NIPPV was used by 7.2% of the patients, and 29.3% were under TPPV. The clinical profiles of the patients with TPPV were shown in Table 4. Mean duration from introduction of TPPV was 3.7 years, and 42.2% of the patients with TPPV were living under home care.

3.2. Age at onset influences progression of disease assessed by duration from onset to introduction of TPPV

The mean interval between the onset of disease and the introduction of TPPV was 3.0 years. Intervals from the disease onset to the introduction of TPPV became shorter as the age at onset advanced (Fig. 1A). There was a significant correlation between the

Table 3
Nutritional and respiratory support of patients registered in 2005 (n=4202)

Nutritional and respiratory support	Frequency (%)
Tube feeding	
Gastrostomy tube	28.7
Nasogastric tube	7.8
NIPPV [a]	
Intermittent use	2.0
All-night use	2.6
All-day use	2.6
TPPV [b]	29.3

[a] Non-invasive positive pressure ventilation.
[b] Tracheostomy positive pressure ventilation.

Table 4
Clinical profiles of patients with TPPV (n=2128)

Male/female (%)	59.9/40.1
Age at onset (years, mean±SD)	59.8±11.7
Duration of disease (years, mean±SD)	6.7±5.0
Duration from disease onset to introduction of TPPV	3.0±3.2
Duration from TPPV introduction	3.7±3.5
Living conditions	
Under home care (%)	42.2[a]
In hospital (%)	57.4[a]
In nursing-care facility (%)	2.1

[a] 1.8% of patients overlap.

age at onset and the interval from disease onset to introduction of tube feeding or TPPV, when analyzed using Pearson's correlation coefficient (r=−0.39 p<0.001). Since 65 years was the mean age of onset, we assessed the cumulative frequency of TPPV in subgroups of patients with an age at onset of 65 years or more and less than 65 years, showing that the duration from onset to introduction of TPPV was significantly shorter in patients with an onset age of 65 years or older (p<0.001) (Fig. 1B). The age at onset influences the progression from onset to the advanced stage assessed by the introduction of TPPV.

3.3. Appearance of ophthalmoplegia under TPPV influenced by age at onset

In the patients with long-standing TPPV, rare symptoms such as ophthalmoplegia were frequently observed. Ophthalmoplegia, which is particularly well assessed by bedside examination, was seen in only

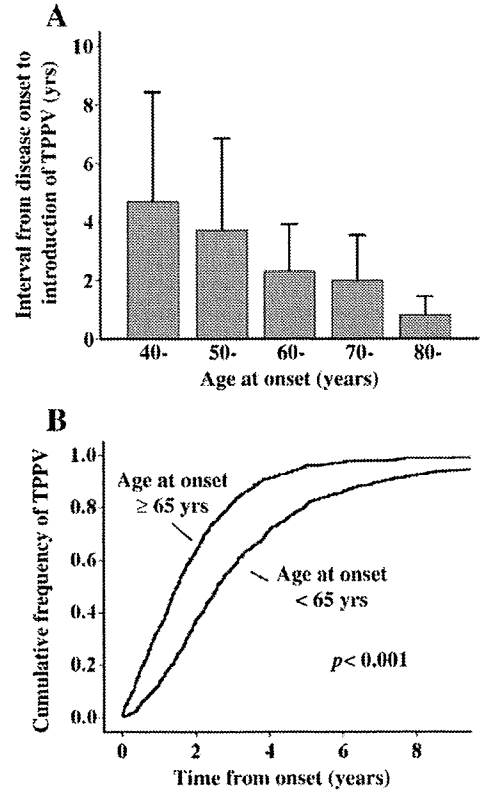

Fig. 1. Relationship between age at onset and introduction of tube feeding and TPPV. Interval from disease onset to introduction of TPPV (A) is shown. An older age at onset strongly correlates to shorter intervals from onset to TPPV. Cumulative frequencies of patients with TPPV in the patient population with an onset age older or younger than 65 years are shown (B). Cumulative curves for patients with an onset age of 65 years or more show significantly shorter intervals between disease onset and introduction of TPPV than those with an onset age of under 65 years of age, suggesting that age at onset markedly influences the time from onset to introduction of TPPV. n=2128.

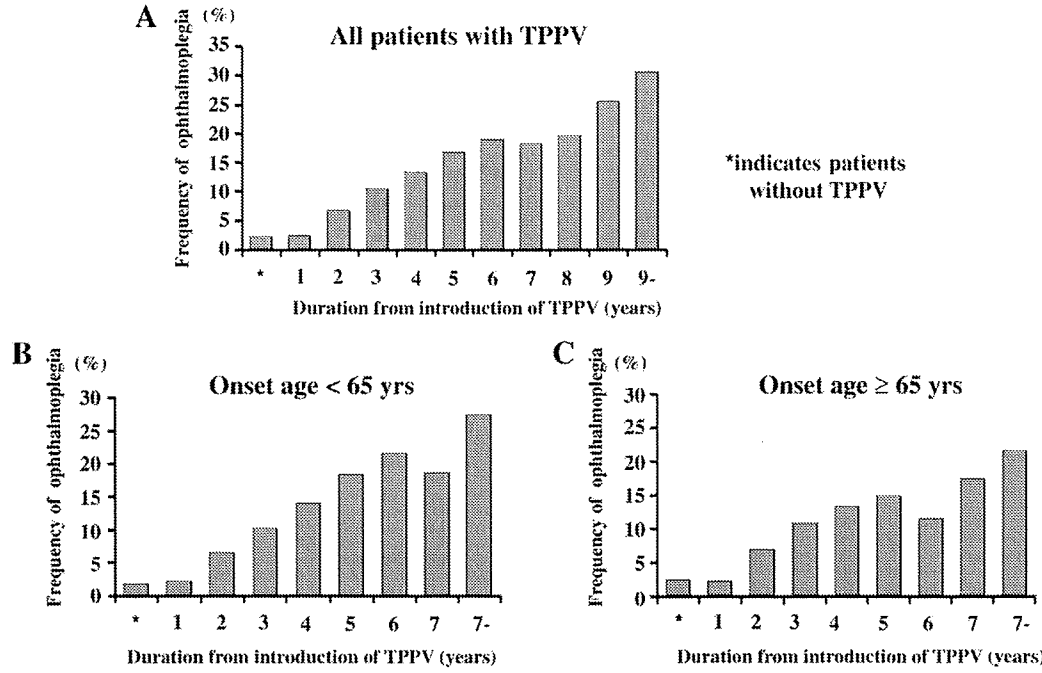

Fig. 2. Frequency of ophthalmoplegia in patients under TPPV, in terms of duration of TPPV and the influence of onset age on its appearance. Ophthalmoplegia rarely occurs in patients without TPPV (*), while its occurrence gradually increases with advanced duration of TPPV (A). Following 9 years of TPPV, almost 30% of patients show ophthalmoplegia. Frequencies of ophthalmoplegia in the patient population with onset age older or younger than 65 years are shown in B and C. Ophthalmoplegia is less frequent in patients with an age at onset of 65 years or older (C). The total frequency of ophthalmoplegia in the patients with onset age older than 65 years or younger than 65 years is 8.3% and 15.1%, respectively. A significant difference exists between them by the chi-square test ($p<0.001$). $n=2128$.

2.0% of the patients without TPPV. The frequency of ophthalmoplegia was increased with the advanced duration of TPPV (Fig. 2A). However, ophthalmoplegia was observed in 30% of patients under TPPV for more than 9 years.

The appearance of ophthalmoplegia under long-standing TPPV is also influenced by the age at onset (Fig. 2B,C). The patients with an age at onset under 65 years showed a higher frequency of appearance of oculomotor symptoms than those with an age at onset over 65 years (Fig. 2B,C). The total frequency of ophthalmoplegia in the patients under TPPV with an onset age of older than 65 years or younger than 65 years was 8.3% and 15.1%, respectively. A significant difference was found between them by the chi-square test ($p<0.001$). These observations suggest that a younger age at onset advances the appearance of ophthalmoplegia compared to patients with an older age at onset. The average time from onset to introduction of TPPV was, however, 1.86± 1.70 years in the patients with an onset age over 65 years, and 3.60± 3.72 years in those with an onset age of younger than 65. This difference influenced the appearance rate of ophthalmoplegia.

3.4. Age at onset influences the frequency of initial symptoms

We analyzed the relationships between the age at onset and the initial symptoms. Dysarthria and dysphagia as the initial symptoms were markedly increased in patients with an advanced age at onset

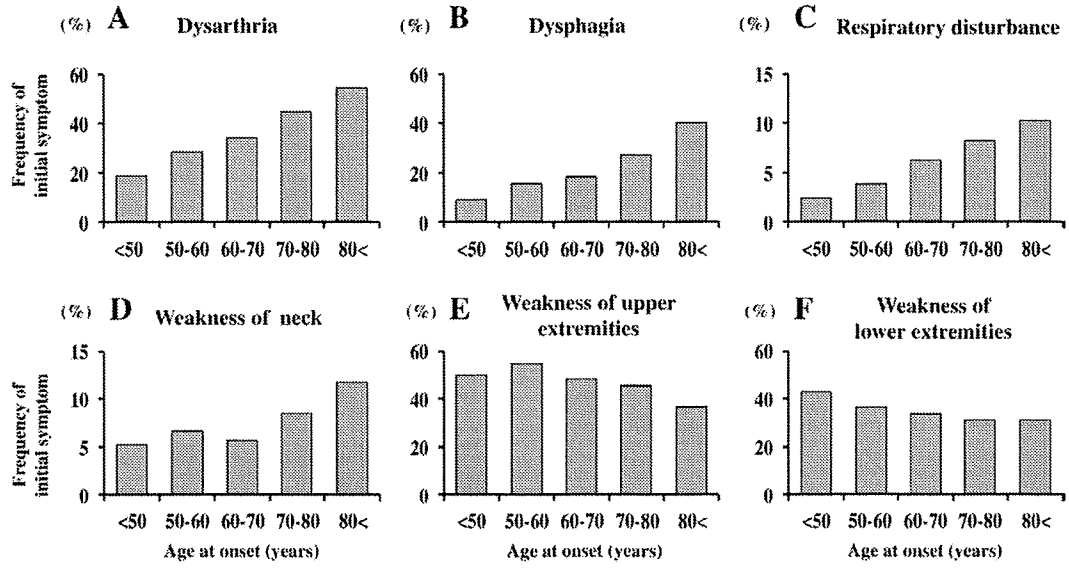

Fig. 3. Age at onset and frequency of initial symptoms. Dysarthria (A), dysphagia (B), respiratory disturbance (C) and weakness of neck (D) are increased in frequency as an initial symptom as the age at onset increases. In contrast, weakness of the upper extremities (E) or lower extremities (F) decreased as the onset age increases. $n=3428$.

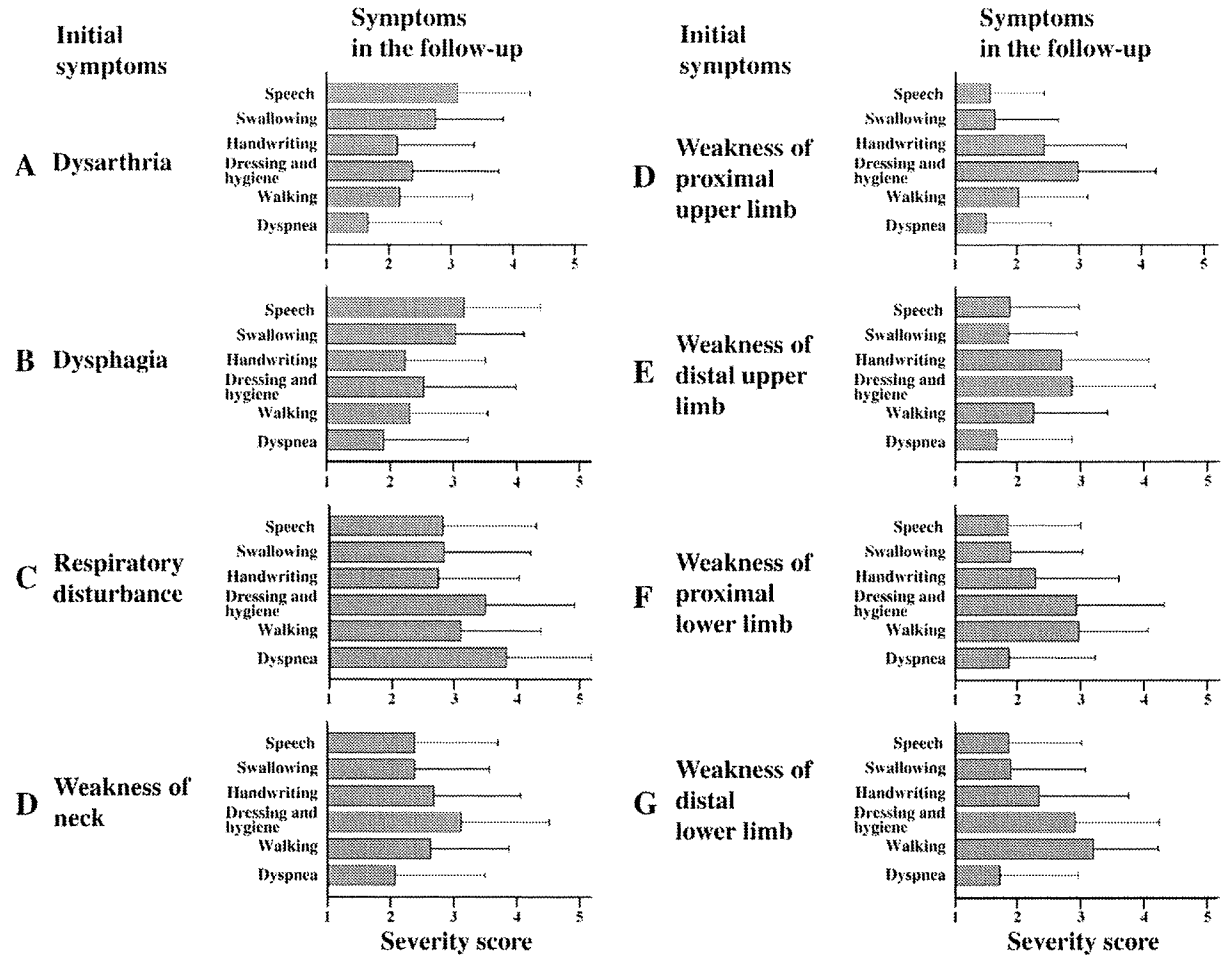

Fig. 4. Relationship between initial symptoms and symptoms at the follow-up stage. Severity scores of Speech, Swallowing, Handwriting, Dressing and Hygiene, Walking and Dyspnea are shown as subscales of ALSFRS-R. The score of "5" represents the most severe state, and "1" represents the absence of the symptom. Initial symptoms remain the most prominent or related symptoms even in the follow-up stage for 1.7±2.2 years from onset, suggesting that initial symptoms significantly determine the prominent features of symptoms throughout the disease course. $n=3428$.

(Fig. 3A,B). On the other hand, weakness in the upper or lower limbs as an initial symptom was seen more frequently in patients with a younger age at onset, and these frequencies gradually decreased with increasing age at onset. As for the respiratory disturbance and dropping head due to weakness of the neck muscles, the frequencies increased gradually with increasing age at onset. When we divided the patients between those with an onset age of older than 65 years and those younger than 65 years and analyzed the data with the chi-square test, the differences in frequencies of dysarthria, dysphagia, respiratory disturbance, weakness of upper extremities and weakness of lower extremities as initial symptoms were also significant between those groups ($p<0.001$, $p<0.001$, $p<0.001$, $p=0.001$, $p=0.019$, respectively). The difference in the frequency of neck weakness was not significant ($p=0.07$), although the tendency was apparent, and may be due to the small number of patients with neck weakness as an initial symptom. These observations suggest that age at onset is a determining factor of the features of the initial symptoms. Correlations between age at onset and the frequency of initial symptoms were similarly observed in the male and female patient groups (Supple. Fig. 1).

3.5. Initial symptoms determine major clinical features in follow-up stage

We examined the relationship between the initial symptoms and the symptoms assessed by 6 items of ALSFRS-R at examination at 1.7±2.2 years after the onset (Fig. 4). At the follow-up stage, the patients who showed a bulbar symptom as an initial symptom showed speech or swallowing disturbance as a major symptom in the follow-up stage. Patients that showed respiratory disturbance as an initial symptom also showed dyspnea as the most prominent disturbance; patients with weakness of distal upper limb muscles showed the most prominent disturbance in handwriting and dressing; patients with weakness of proximal upper limbs showed prominent disturbance in dressing and hygiene; and patients with weakness of lower limbs, either proximal or distal, all showed a prominent disturbance in walking. These observations strongly suggested that the initial symptoms remained the most prominent or related symptoms even in the follow-up stage, and support the view that the initial symptoms determine the clinical features of the individual patient even in the follow-up stage. A similar tendency was observed in the male and female patient groups (Supple. Fig. 2).

4. Discussion

The results of the present study demonstrate the characteristic clinical profiles of Japanese sporadic ALS patients. A very high rate of Japanese ALS patients (29.3%) were under TPPV compared to patients in North America or Europe [10,11,17,18] which are 2.1–5.4%, respectively. The frequency of patients showing rare symptoms such as ophthalmoplegia increased with disease progression, particularly under long-standing TPPV.

A striking observation in the present study is that the age at onset greatly influences the wide-ranging clinical features, including the initial symptoms, progression to the endstage assessed by introduction of TPPV, and the frequency of rare symptom in the long-standing course. A higher incidence of bulbar involvement in patients with an older age at onset has been reported in some previous studies [19–23]. We extended these observations in that almost all of the initial symptoms, such as dysphagia, dysarthria, upper or lower limb weakness, respiratory failure and head dropping are strongly influenced by the age at onset. This observation was also confirmed in the subpopulation of male and female patients. In addition, since the initial symptoms also determine the prominent clinical phenotypes in the follow-up stage as demonstrated in this study, age at onset may influence not only the initial symptoms, but also the entire clinical phenotypes of sporadic ALS. The underlying mechanism for the onset age influence on the initial manifestation of the symptoms is unknown. Furthermore, we do not know the mechanism by which patients with a younger age at onset tend to show a higher frequency of rare symptoms. Further study is needed to resolve these issues, although one may speculate that subpopulations of the motor neurons may be differentially vulnerable to the aging process. In several sporadic neurodegenerative diseases, age at onset has been suggested to be an influencing factor for the spatial development of neural involvement, and, thus, for the features of clinical manifestations [24]. In Parkinson's disease, for instance, patients with an older age at onset have been suggested to have a tendency to show a higher cognitive dysfunction and autonomic dysfunction [25–27], whereas, those with a younger age at onset have an increased tendency toward dystonia and a diurnal fluctuation of symptoms [28,29]. Taking these observations together with our findings on ALS, age at onset may be a more important factor modifying clinical manifestations in sporadic neurodegenerative diseases than previously thought.

Age at onset also influenced the interval from the onset to the time of introduction of TPPV. Reserved respiratory function is known to decrease with advancing age [19]. Therefore, the short interval between the onset and the introduction of TPPV may be explained by the smaller reserved respiratory capacity in elderly patients. Indeed, serial examinations of the respiratory function in elderly patients start at a lower vital capacity and reach a critical point more quickly than younger patients [19,30]. It is congruent with the fact shown in the previous reports [1,3,5,6,22], that younger ALS patients survive longer than older patients.

Therefore, in taking into account the age at onset, initial symptoms, occurrence of rare symptoms and progression, the age at onset greatly affects the clinical profiles of sporadic ALS patients. In addition, the onset age-related initial symptoms are important to estimate the patient's prognosis as well as the design of clinical trials [31].

A high proportion of ALS patients in Japan are under TPPV compared to patients in other countries, possibly for social, cultural and economic reasons [13,17,18]. The presence of a subgroup of patients extending involvement to other systems beyond motor neurons, such as oculomotor, autonomic, sensory and higher functional systems, has been described in Japanese ALS patients under long-term TPPV treatment [32–36]. Pathologically, these patients show an extensive involvement of the tegmentum of the brainstem, substantia nigra, Clarke's dorsal nuclei and spinocerebellar tract, and frequent involvement of the thalamus and globus pallidus. Our present observations have confirmed these reports on sporadic Japanese ALS patients, particularly those with long-standing TPPV, and demonstrated that these subpopulations with a rare extension of involvements include almost 30% of the patients with 9 years or more under TPPV, particularly those assessed for oculomotor system involvement. However, further studies are needed to determine whether all the patients would eventually show an extended involvement beyond the motor system or whether these patients with an extended form are restricted to a given subpopulation. This is an important issue to determine the natural history of sporadic ALS. Since European and American ALS patients are not generally maintained on TPPV treatment for a longer period as Japanese patients, extended involvement is very rarely observed in Europe or North America.

In summary, we have presented the clinical profiles of sporadic Japanese ALS patients based on a large-scale sample. As demonstrated, age at onset may be a remarkable factor influencing wide-ranging clinical profiles including the progression and prognosis. We should take account of this observation in cohort studies or clinical trials.

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Appendix A

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Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jns.2008.09.024.

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Screening for *TARDBP* mutations in Japanese familial amyotrophic lateral sclerosis

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ABSTRACT

TAR-DNA-binding protein 43 (TDP-43), encoded by the *TARDBP* gene on chromosome 1p36.22, has been identified as the major pathological protein in abnormal inclusions in neurons and glial cells in sporadic amyotrophic lateral sclerosis (SALS), *SOD1*-negative familial ALS (FALS) and frontotemporal lobar dementia (FTLD). Twenty mutations of *TARDBP* in *SOD1*-negative FALS and SALS cases have been reported so far. To investigate the presence and frequency of *TARDBP* mutations in Japanese *SOD1*-negative FALS patients, we performed mutational screening of *TARDBP* in 30 *SOD1*-negative FALS patients. An N352S mutation was found in one case of FALS, but no *TARDBP* mutations were found in cases of SALS. It was thought that this mutation increases TDP-43 phosphorylation. This might lead to impaired nuclear cytoplasmic transport or protein–protein interaction, thereby leading to TDP-43 accumulation.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease. It is a progressive disorder that involves degeneration of upper and lower motor neurons at all levels of the motor system, from the cortex to the anterior horn of the spinal cord. The clinical features of ALS can be considered in relation to neurological regions or levels (bulbar, cervical and lumbar). The disorder is characterized by dysarthria, dysphagia, brisk reflexes, pyramidal signs, fasciculation, and progressive atrophy and muscle weakness. The mean duration of survival is three to five years from onset without intensive physiological support (e.g., a ventilator) [1,2]. About 10% of cases of ALS are familial (FALS), and the others are thought to be sporadic (SALS) [3–5]. Various genes that cause FALS, including copper/zinc superoxide dismutase-1 (*SOD1*) [6], dynactin 1 [7–9], alsin [10], senataxin [11], vesicle-associated membrane protein B [12] and angiogenin [13,14], have been identified, but the frequency of their mutation is low.

TAR-DNA-binding protein 43 (TDP-43) has recently been identified as the major pathological protein in abnormal inclusions in neurons and glial cells in SALS, *SOD1*-negative FALS and frontotemporal lobar dementia (FTLD) [15–17]. Some reports suggest clinical and pathological overlap between ALS and FTLD [18–20]. TDP-43 is encoded by *TARDBP* on chromosome 1p36.22, and its structure is evolutionarily conserved, consisting of two RNA recognition motifs and a glycine-rich domain. It was originally identified as a transcriptional receptor that binds to the TAR-DNA element of human immunodeficiency virus type 1 (HIV-1) [21]. TDP-43 is involved in the regulation of expression and splicing, and it is part of a complex that splices the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) [21–25]. In ALS, 20 mutations of *TARDBP* have been reported not only in *SOD1*-negative FALS cases (G290A, G298S, A315T, M337V, Q343R, N345K, N352S, A382T, I383V) but also in SALS cases (D169G, G287S, G294A, Q331K, G348C, R361S, P363A, Y374X, A382P, N390D, N390S) [26–33].

In this study, in order to investigate the presence and frequency of *TARDBP* mutations in Japanese *SOD1*-negative FALS patients, we performed mutational screening of *TARDBP* in *SOD1*-negative FALS patients, SALS patients and healthy control subjects.

2. Materials and methods

The subjects included 30 *SOD1*-negative FALS patients from 30 unrelated families (mean age at onset, 60.2 years; age range, 33–76 years), 220 (including 12 autopsy-confirmed) SALS patients (mean age at onset, 58.6 years; age range, 23–84 years), and 105 healthy control subjects (mean age, 63.9 years; age range, 40–96 years). All of

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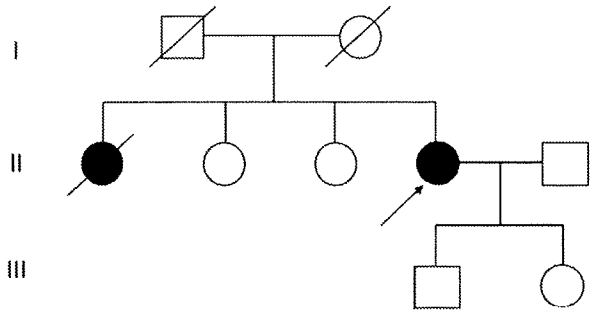

Fig. 1. Pedigrees of Japanese familial ALS with N352S TARDBP mutations. Black symbols represent patients affected with ALS. White symbols represent unaffected individuals.

the subjects were Japanese. Informed consent for participation in this study was obtained from all subjects.

Genomic DNA was extracted from peripheral blood leukocytes or frozen brain sections using standard methods. In cases of FALS, the entire coding region of the *TARDBP* gene (accession number NM_007375), consisting of exons 2–5 and the first 531 nucleotides of exon 6, was amplified with primers designed using Primer3 software. In SALS patients and healthy control subjects, only the first 531 nucleotides of exon 6 were amplified because exon 6 seems to be a hotspot for ALS-linked *TARDBP* mutations [26–33]. Each PCR product was sequenced using Applied Biosystems BigDye terminator v3.1 sequencing chemistry and run on an ABI PRISM 3130 Genetic Analyzer.

3. Results

A c.1055 A>G mutation, predicted to substitute asparagine for serine at codon 352 (p.N352S), was identified in one case of FALS (Fig. 1). This mutation was not found in any of the 200 SALS patients or the 105 healthy subjects. The patient with this mutation first showed clinical signs at the age of 55 years, beginning with weakness of the right hand. The symptom was progressive. Gait disturbance, bulbar signs and respiratory impairment appeared 2 years later. Cognitive function was normal. Electromyography showed acute and chronic changes in the upper and lower limbs and cranial lesions. The patient's older sister had been bedridden with respiratory impairment and died of ALS at the age of 42 years. The patient's father died of an accident and her mother died of stroke.

We also found a c.1098C>G variation (p.A366A) in 16 cases of SALS. However, this variation was silent and was thought to be a benign polymorphism as it was also found in seven control subjects.

4. Discussion

In this study, we found an N352S missense mutation in *TARDBP* in a patient with *SOD1*-negative FALS. This mutation was previously reported in a German family [27]. The frequency of the *TARDBP* mutation was 3.3% (1 of 30 patients). The frequency of the *TARDBP* mutation in *SOD1*-negative FALS patients in previous studies was 0.6% to 6.5% [26–32]. In Japan, Yokoseki et al reported one missense mutation (p. Q343R) in 16 *SOD1*-negative FALS patients [31]. Combining the number of *TARDBP* mutations in Japanese *SOD1*-negative FALS yields a rate of 2 in 46 (4.3%). Our identified mutation was not present in our SALS and healthy control subjects.

In previous studies, the clinical phenotype of *TARDBP* mutation cases consisted mainly of spinal onset and absence of cognitive impairment [26–32]. The clinical phenotype in our case was similar. This clinical phenotype does not allow for separating *TARDBP* mutation cases from other forms of ALS, with similar features being reported in SALS and in *SOD1* FALS [34].

TDP-43-positive FALS is thought to be an autosomal dominant trait. In this family, the parents did not show ALS symptoms. The father or mother might have had the same mutation but died before ALS onset, or the mutation might have had low penetrance. De novo mutation is also a possibility. However, this seems unlikely since the patient's older sister also had ALS.

Except for the D169G mutation, all other *TARDBP* mutations are located in exon 6 encoding for the C-terminus of TDP-43. Mutations of the C-terminus region of TDP-43 may impair the function or transport of TDP-43 by influencing protein–protein interaction, transport through the nuclear pore, or exon skipping and splicing inhibitory activity. These *TARDBP* mutations may also cause a toxic gain of function through novel protein interactions or intracellular accumulation of TDP-43 fragments, leading to apoptosis [26–32].

The N352S mutation is localized to a highly conserved region of the C-terminus of TDP-43 that is known to be involved in protein–protein interaction. Asparagine at codon 352 is conserved across all mammals examined so far, as well as in *Gallus gallus* [27]. Kuhnlein et al. predicted that the most likely effect of the N352S mutation might be an increase in TDP-43 phosphorylation and that the N352S mutation might not only introduce a new serine residue at position 352 but also lead to an increase in the phosphorylation prediction score for serine residues at positions 347 and 350 of TDP-43 using a network service [27]. This might lead to impaired nuclear cytoplasmic transport or protein–protein interaction, resulting in TDP-43 accumulation.

In conclusion, we identified one *TARDBP* mutation in *SOD1*-negative FALS. The frequency of *TARDBP* mutations in FALS may not be high compared with the frequency of SOD1 mutations, but the function analysis of *TARDBP* mutations may contribute to understanding the cause of ALS because TDP-43 is the major pathological protein in the abnormal inclusions of ALS. The identification of rare familial mutations in the β-amyloid precursor protein in Alzheimer's disease and in α-synuclein in Parkinson's disease has dramatically advanced studies aimed at elucidating the pathogenesis of predominantly sporadic diseases. Further studies, including studies using transgenic animal models, are needed to elucidate the links between TDP-43 amino acid change, TDP-43 neuropathology, and ALS neurodegeneration.

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