opening duration as the secondary end point of this trial and measured other parameters as references. All the parameters were measured blindly by two independent evaluators according to standard procedures.[32,33] In brief, duration of cricopharyngeal opening was defined as the length of time during which the cricopharyngeal sphincter was open. Pharyngeal delay time was defined as the interval from the bolus passing the base of the tongue to the onset of laryngeal elevation, whereas duration of maximum laryngeal elevation was the length of time during which the larynx was maximally elevated from its rest position. Pharyngeal residue was measured using semiquantitative scales: 0, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%.

Immunohistochemical Detection of Mutant Androgen Receptor

Immunohistochemistry of scrotal skin (from biopsies), spinal cord, and pontine base (from autopsies) specimens were conducted as described previously.[16,17] In brief, 6μm-thick, formalin-fixed, paraffin-embedded sections were prepared, deparaffinized, rehydrated, and pretreated by immersing in 98% formic acid for 5 minutes and then microwaving for 15 minutes in 10mM citrate buffer at pH 6.0. Sections were incubated with a mouse antiexpanded polyglutamine antibody (1C2; 1:20,000; Chemicon, Temecula, CA)[34] to evaluate the nuclear accumulation of mutant AR.[16,20,21] Immune complexes were visualized using the Envision-plus kit (Dako, Glostrup, Denmark). Sections were counterstained with Mayer's hematoxylin. Quantitative assessment of 1C2-positive cells in scrotal skin was performed as described previously.[17] In brief, the frequency of diffuse nuclear staining was calculated from counts of more than 500 nuclei in 5 randomly selected fields of each section (BX51TF; Olympus, Tokyo, Japan). To assess the nuclear accumulation of mutant AR in spinal cord motor neurons, we prepared at least 100 serial transverse sections from the cervical spinal cord and immunostained every 10th section with the anti-polyglutamine 1C2 antibody. For the purposes of counting, a neuron was defined by the presence of its obvious nucleolus in a given 6μm-thick section. The numbers of 1C2-positive and -negative cells within the ventral horn on both the right and left sides were counted under the light microscope with a computer-assisted image analyzer (BX51TF; Olympus), as described previously.[16,35,36] For quantification of 1C2-positive neurons within the pontine base, the frequency of diffuse nuclear staining was calculated from counts of more than 500 neurons in a total of 50 or more fields from each section (BX51N-34; Olympus), as described previously.[37] Populations of 1C2-positive cells were expressed as percentages of the total cell counts.

Autopsy Study

Autopsy specimens of cervical spinal cord (seven patients) and pons (five patients) were obtained from nine control, genetically confirmed SBMA patients who had not participated in any therapeutic trials (52–83 years old; men; 41–52 CAG repeats) and one subject (70 years old) who died at week 67 of the 96-week follow-up study (Patient 16), who had been allocated to the leuprorelin group in the 48-week RCT and had continued leuprorelin administration in the 96-week follow-up trial. The last administration of leuprorelin acetate was at week 60 of the follow-up trial. The causes of death of the control patients were pneumonia in three, respiratory failure in three, unknown in two, and lung cancer in one. Immunohistochemistry of the specimens was performed as described earlier. The collection of tissues and their use for this study were approved by the Ethics Committee of Nagoya University Graduate School of Medicine.

Genetic Analysis

Genomic DNA was extracted from peripheral blood of the patients using conventional techniques, and the CAG repeat size was determined as described previously.[9,11,38] In brief, polymerase chain reaction amplification of the CAG repeat in exon 1 of the *AR* gene was performed using a fluorescein-labeled forward primer (5′-TCCAGAATCTGTTCCAGAGCGTGC-3′) and a nonlabeled reverse primer (5′-TGGCCTCGCTCAGGATGTCTTTAAG-3′). Size of the CAG repeat was analyzed using Fraglys software version 2.2 (Hitachi Electronics Engineering, Tokyo, Japan) by comparison with coelectrophoresed polymerase chain reaction standards with known repeat sizes. Patients with 38 or more CAGs were diagnosed with SBMA.[10] All patients gave their written informed consent to genetic analyses.

Statistical Analyses

The effectiveness analysis and safety evaluation were conducted on data from the intention-to-treat population in the 48-week RCT. We analyzed the data by Pearson's coefficient, Spearman's rank correlation, and Student's t test. The Mann–Whitney U test was used to analyze serum testosterone levels. p values less than 0.05 were considered indicative of significance. For multiple comparisons, p values were corrected using the Dunnett test. Computations were performed with SPSS software (version 14.0J for Windows; SPSS Japan, Tokyo, Japan).

Ethics

This study was conducted according to the Declaration of Helsinki (Hong Kong Amendment). Written informed consent was obtained from each patient. Patients were free to withdraw from the study at any time for any reason. The protocol was approved by the Nagoya University Hospital Institutional Review Board. Confidentiality was ensured by assigning a study code to each patient. All studies conformed to the ethics guidelines for human genome/gene analysis research and the ethics guidelines for epidemiological studies endorsed by the Japanese government.

Results

Demographics

Fifty participants met the eligibility criteria, gave informed consent, and were assigned to either the leuprorelin or placebo group. There were no significant differences in the characteristics of the two groups (Table 1). There were no protocol deviations, although one patient in the leuprorelin group discontinued the drug after 16 weeks because of the patient's schedule, but this patient was included in the end-point analyses.

Table 1. **Characteristics of Patients in the 48-Week Randomized Controlled Trial (RCT)**

Characteristics	Leuprorelin (n = 25)	Placebo (n = 25)	*p*
Mean age ± SD, yr	52.8 ± 7.4	52.0 ± 8.9	NS
Mean height ± SD, cm	167.5 ± 6.2	168.1 ± 6.1	NS
Mean weight ± SD, kg	58.4 ± 5.7	60.2 ± 6.2	NS
Mean duration of weakness ± SD, yr	10.8 ± 6.3	12.9 ± 8.2	NS
Mean (CAG)n ± SD	48.5 ± 3.2	48.1 ± 2.5	NS
Mean ALSFRS-R score ± SD (Japanese edition)	41.1 ± 3.7	42.0 ± 3.4	NS
ADL (cane-assisted/independent)	6/19	7/18	NS

SD = standard deviation; NS = not significant; (CAG)n = number of expanded CAG repeats in the *androgen receptor* gene; ALSFRS-R = revised amyotrophic lateral sclerosis functional rating scale; ADL = activities of daily living.

No patients discontinued treatment prematurely because of adverse events during the 48-week RCT. At the end of the 48-week RCT, 34 of the 50 patients elected to receive leuprorelin administration in the follow-up trial before the key was broken. During this 96-week follow-up trial, one patient discontinued treatment mainly because of depression but was followed up without leuprorelin administration. One patient (Patient 16) died of acute cardiac failure at week 67 and was not included in the end-point analyses (see Fig 1).

Forty-eight-Week Randomized Controlled Trial

The outcome measures of the 48-week RCT are shown in Figure 2. In patients who received leuprorelin acetate, serum testosterone levels decreased to near zero within 4 weeks of the treatment (see Fig 2A). In the placebo group, ALSFRS-R scores had declined by 0.9 point at week 48, suggesting that the change in motor function of patients in this trial was similar to that in a previous study on the natural history of SBMA.[39] Although there was no significant difference in the changes in ALSFRS-R *total* scores at week 48 in the leuprorelin and placebo groups (see Fig 2B), there was a tendency for the swallowing subscores to be improved in the leuprorelin group (see Fig 2C). This view was supported by the fact that the cricopharyngeal opening duration was significantly extended in the leuprorelin group compared with the placebo group, suggesting that androgen deprivation suppressed deterioration of swallowing function in SBMA ($p < 0.05$; see Fig 2D). The serum level of creatine kinase, a marker of muscular involvement in SBMA, and those of liver enzymes also tended to be decreased in the leuprorelin group (see Fig 2E; see Supplemental Table 3). Diffuse nuclear staining was predominantly observed in the scrotal skin biopsy. The frequency of 1C2-positive cells in the scrotal epithelium was significantly decreased at week 48 in the leuprorelin group ($p < 0.001$; see Fig 2F). There were no significant effects of leuprorelin acetate on all other secondary end points (see Supplemental Table 3). Although we performed stratified analyses, neither CAG repeat size nor age had any influence on the outcome measures (data not shown).

Ninety-six-Week Follow-up Trial

All but one patient, who discontinued treatment early in the 48-week RCT, underwent an additional 96-week follow-up. Fifteen patients declined to continue leuprorelin administration mostly because of economic reasons. As shown in Table 2, at the time of enrollment in the follow-up trial, there were no differences in the characteristics of patients who participated and those who were not enrolled, indicating no selection bias for the enrollment.

In the follow-up trial, we compared ALSFRS-R scores and VF findings of the following groups: Group A—patients who were allocated to the leuprorelin group for 48 weeks and received leuprorelin for an additional 96 weeks; Group B—patients who were allocated to the placebo group and received leuprorelin for an additional 96 weeks; Group C—patients who were allocated to the leuprorelin group for 48 weeks but did not receive treatment during the 96-week follow-up; and Group D—patients who were allocated to the placebo group and were followed up without leuprorelin treatment for 96 weeks. Multiple comparisons were performed with Group D as the control. We did not include the following two subjects in these analyses: one patient in Group A who died during the follow-up period and one in Group C who was diagnosed with multiple myelomas during the follow-up period. At week 96 of the follow-up trial, ALSFRS-R scores were significantly greater in Groups A and B than in Group D (Figs 3A, B). Similarly, the swallowing subscores of the ALSFRS-R were significantly greater in Group A than in Group D (see Fig 3C). Cricopharyngeal opening duration in VF was also significantly longer in Groups A and B than in Group D (see Fig 3D).

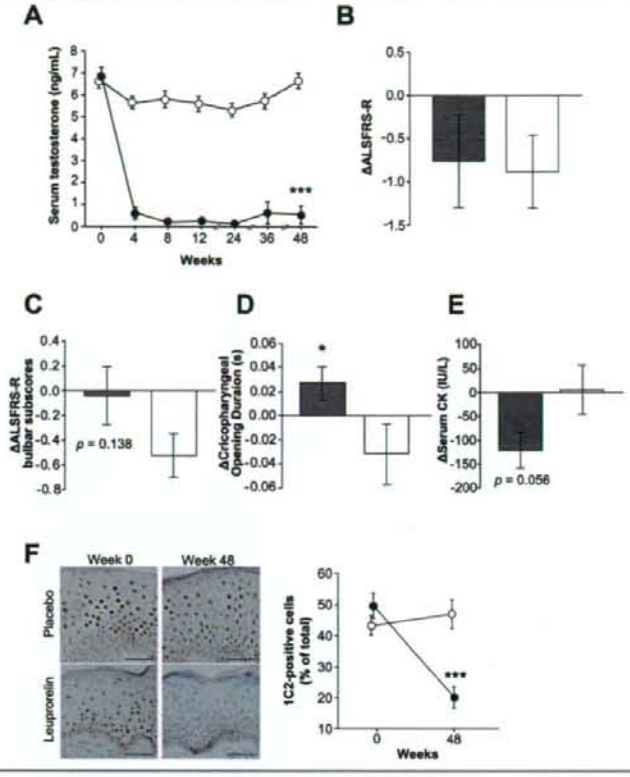

Fig 2. Efficacy results of the 48-week randomized controlled trial (RCT). (A) Treatment with leuprorelin acetate (black circles) *rapidly depleted serum testosterone levels.* White circles *represent placebo group. (B) There was no significant difference in the change in Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) score between the groups. (C) There was a favorable tendency in the swallowing subscores of the ALSFRS-R in the leuprorelin group. (D) Cricopharyngeal opening duration was significantly extended by the 48-week leuprorelin treatment. (E) Serum creatine kinase (CK) levels also tended to be decreased in the leuprorelin group. (F) The frequency of diffuse nuclear 1C2 staining (indicative of mutant androgen receptor [AR]) in the scrotal epithelial cells was significantly decreased after the 48-week administration of leuprorelin acetate.* White bars *represent placebo group;* black bars *represent leuprorelin acetate group. Scale bars = 50μm. Data are expressed as means ± standard error of the mean.* $^{*}p < 0.05$; $^{***}p < 0.001$.

Safety and Tolerability

There were a total of 58 adverse events recorded during the 48-week RCT; none was so serious as to require hospitalization (Table 3). The most frequent adverse event in the leuprorelin group was a loss of sexual function, recorded as erectile dysfunction, but this symptom was also often seen in the placebo group, suggesting androgen insensitivity in SBMA patients. Although increases in total cholesterol, triglyceride, fasting blood sugar, or glycosylated hemoglobin (HbA1c) were seen in the leuprorelin group, no marked exacerbations were observed. The details of adverse events during the 96-week follow-up trial were obtained from Groups A, B, and D. As shown in Table 4, there were no treatment duration–dependent adverse effects of leuprorelin acetate as reported previously.[40]

Autopsy Study

One participant (Patient 16) who received leuprorelin acetate in the 48-week RCT and continued to receive leuprorelin acetate in the 96-week follow-up trial died 118 weeks after initiation of the treatment. Autopsy of the patient indicated acute cardiac failure caused by cardiac arrhythmia as a possible cause of death. Otherwise, no specific causes of death were reported. Autopsied specimens were assessed by anti-polyglutamine (1C2) immunohistochemistry as in the scrotal skin biopsy and were compared with the findings of previously autopsied SBMA cases who had not been treated with leuprorelin acetate or with relevant drugs. In the spinal motor neurons, diffuse nuclear staining of 1C2 was predominantly observed, and nuclear inclusions were less frequent. The frequencies of 1C2-positive

Table 2. **Characteristics of Patients in the 96-week Follow-up Trial**

Treatment in 96-week Follow up	Leuprorelin in 48-week RCT (n = 22)			Placebo in 48-week RCT (n = 25)		
	Leuprorelin (Group A, n = 18)	No Treatment (Group C, n = 4)	*p*	Leuprorelin (Group B, n = 15)	No Treatment (Group D, n = 10)	*p*
Age (yr)	52.0 ± 6.5	56.3 ± 8.1	NS	52.5 ± 8.2	51.3 ± 10.2	NS
Height (cm)	168.6 ± 5.8	164.3 ± 9.4	NS	168.5 ± 5.0	167.6 ± 7.7	NS
Weight (kg)	58.6 ± 5.9	58.8 ± 6.2	NS	59.6 ± 5.6	61.2 ± 7.3	NS
Duration of Weakness (yr)	11.7 ± 6.4	8.3 ± 7.4	NS	12.8 ± 5.5	13.0 ± 11.5	NS
(CAG)n	49.1 ± 3.3	45.8 ± 2.2	NS	48.0 ± 2.5	48.2 ± 2.6	NS
ALSFRS-R (Japanese Edition) [a]	41.3 ± 2.8	40.5 ± 7.7	NS	42.1 ± 2.6	42.0 ± 4.5	NS
	41.2 ± 3.7	39.8 ± 6.7	NS	40.7 ± 3.6	41.9 ± 5.0	NS
ADL (cane-assisted/independent) [a]	3/15	1/3	NS	4/11	3/7	NS
	7/11	2/2	NS	6/9	3/7	NS

[a]Upper values indicate data at inclusion in the 48-week RCT, and lower values those at inclusion in the 96-week follow-up trial. Other values are data at inclusion of 48-week RCT. (CAG)n = number of expanded CAG repeats in the *androgen receptor* gene; ALSFRS-R = revised amyotrophic lateral sclerosis functional rating scale. Data represent means ± SD except for ADL.

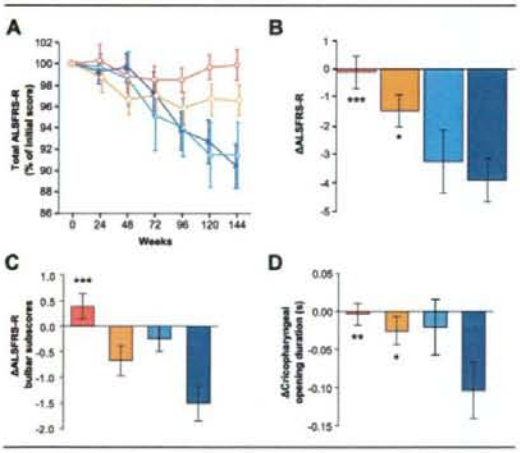

*Fig 3. Efficacy results of the 96-week follow-up trial. (A, B) Changes in the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) scores showed treatment duration–dependent improvements in the leuprorelin-treated groups. (C, D) The ALSFRS-R bulbar subscores (C) and videofluorography (VF) findings (D) were also significantly improved in the leuprorelin-treated patients. Data are expressed as means ± standard error of the mean. *p < 0.05; **p < 0.005; ***p < 0.001 with respect to Group D. Red represents Group A: 48-week leuprorelin/96-week leuprorelin (n = 18); orange represents Group B: 48-week placebo/96-week leuprorelin (n = 15); light blue represents Group C: 48-week leuprorelin/96-week no treatment (n = 4); blue represents Group D: 48-week placebo/96-week no treatment (n = 10).*

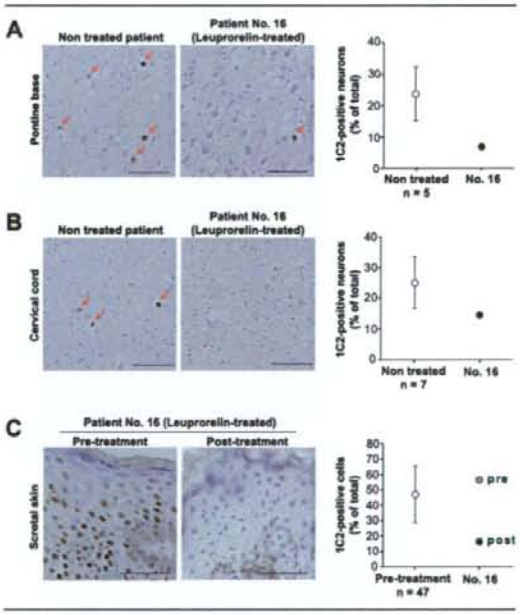

Fig 4. Effects of leuprorelin acetate on nuclear accumulation of mutant androgen receptor (AR). (A, B) Accumulation of mutant AR in neurons was remarkable both in the pontine base and in the spinal anterior horn of all the control, non-treated autopsied cases, but the number of 1C2-positive neurons was relatively small in the leuprorelin-treated patient (Patient 16). Scale bars = 100μm. (C) Mutant AR accumulation in scrotal skin epithelial cells that underwent biopsy was markedly reduced by leuprorelin acetate in Patient 16 (Patient 16 was excluded from this mean.) Scale bars = 50μm. Data are expressed as means ± standard deviation.

Table 3. **Adverse Events in 48-Week Randomized Controlled Trial**

AEs	Leuprorelin (n = 25)	Placebo (n = 25)
At least one AE	21 (84%)	9 (36%)
At least one AE other than ED	16 (64%)	6 (24%)
ED[a]	13 (52%)	4 (16%)
Hypertriglyceridemia	7 (28%)	0
Lumbago	5 (20%)	1 (4%)
Headache	5 (20%)	1 (4%)
Numbness	3 (12%)	2 (8%)
Hand arthralgia	4 (16%)	0
Fatigue	3 (12%)	0
Hot flush	3 (12%)	0
Injection site lump	3 (12%)	0
Hypertension	2 (8%)	0
Fracture	0	2 (8%)

[a]Number was calculated by questionnaire on every visit. AE = adverse event; ED = erectile dysfunction.

neurons in the anterior horn and brainstem of Patient 16 were less than those in non-treated SBMA patients (Figs 4A, B). By way of comparison, the pretreatment frequency of 1C2-positive cells in the biopsied scrotal skin of Patient 16 was a little higher than the mean value of other study participants at week 0 but decreased after 48 weeks of leuprorelin treatment in the RCT (see Fig 4C). Hence, this patient's pretreatment frequency of 1C2-positive cells in the anterior horn and brainstem were presumed to also be greater than the posttreatment levels.[17]

Discussion

Recent research on neurodegenerative diseases has repeatedly shown that abnormal protein accumulation in neuronal cells is important in the molecular pathogenesis of neurodegeneration.[41] In polyglutamine diseases including SBMA, the aberrant proteins that contain an extended polyglutamine tract accumulate chiefly in the nucleus, resulting in the disruption of cellular functions such as transcription.[14,42] To date, no disease-modifying therapies for polyglutamine diseases have proved beneficial in clinical trials. The results of this interventional trial suggest that androgen deprivation therapy for SBMA is a promising therapy targeting the molecular pathogenesis of polyglutamine diseases.

In this study, we demonstrated that leuprorelin acetate suppressed toxic accumulation of the mutant AR protein, and thereby slowed down the progression of SBMA. As shown previously in animal and human studies, leuprorelin-mediated androgen deprivation significantly decreased mutant AR accumulation in scrotal skin.[17,21] Furthermore, our histopathological analysis in the autopsied case suggests that leuprorelin treatment also attenuates the nuclear accumulation of pathogenic AR within neuronal cells. AR did not aggregate even in the cytoplasm of scrotal epithelial cells or in that of spinal motor neurons, presumably because androgen deprivation destabilizes AR and facilitates degradation of the protein.[43] Alternatively, androgen deprivation may enhance the protective effects of heat shock proteins, which are normally associated with AR and dissociate on ligand binding.

The 48-week treatment with leuprorelin acetate significantly extended cricopharyngeal opening duration, indicating that this therapy blocked disease progression measured with the most reliable VF parameter. The opening of the cricopharyngeal sphincter is triggered by the motion of the larynx and is widened by pharyngeal pressure.[44] Therefore, cricopharyngeal opening duration reflects the strength of deglutition and has been used as a quantitative parameter of swallowing function in disease conditions such as stroke and inflammatory myopathy.[45,46] Moreover, in patients with ALS, cricopharyngeal opening duration is shortened as a consequence of delayed opening or premature closure of the cricopharyngeal sphincter, or both, and this shortening correlates well with the severity of dysphagia.[47] The amelioration of dysphagia by androgen deprivation is also supported by the 96-week follow-up trial, in which leuprorelin treatment significantly prolonged cricopharyngeal opening duration and improved the bulbar subscores of the ALSFRS-R. Given that pneumonia and respiratory distress are the main causes of death in this disease, leuprorelin treatment appears to be beneficial for the prognosis of SBMA patients.[7]

Although the effect of leuprorelin acetate on general motor function was not clear in the 48-week RCT, the total ALSFRS-R score was significantly greater in patients who received androgen deprivation therapy for 144 or 96 weeks than in those who received no therapy throughout the trial. Although the total ALSFRS-R score is a reliable marker of the progression of ALS, this score is less sensitive for SBMA.[39,48] This study suggests that the ALSFRS-R score is not an appropriate end point in a short-term trial but may be useful in a long-term clinical trial on SBMA.

No unexpected or serious safety issues associated with the long-term use of leuprorelin acetate were identified during this study. The adverse effects of leuprorelin acetate did not differ from those in trials for prostate cancer.[49,50] Although erectile dysfunction after leuprorelin administration was more frequent in this trial than in previous trials for prostate cancer, this is likely because of pre-existing androgen insensitivity in

Table 4. **Adverse Events during Leuprorelin Administration (48-Week Randomized Controlled Trial and 96-Week Follow-up)**

AEs	Group A (n = 19)[a]	Group B (n = 15)	Group D (n = 10)
At least one AE	18 (95%)	15 (100%)	7 (70%)
At least one AE other than ED	15 (79%)	11 (73%)	5 (50%)
ED[b]	13 (68%)	9 (60%)	2 (20%)
Numbness	7 (37%)	3 (20%)	2 (20%)
Arthralgia	5 (26%)	5 (33%)	1 (10%)
Hot flush	5 (26%)	4 (27%)	0
Injection-site lump	5 (26%)	4 (27%)	0
Lumbago	5 (26%)	1 (7%)	1 (10%)
Myalgia	2 (11%)	2 (13%)	0
Edema	3 (16%)	0	0
Headache	3 (16%)	0	1 (10%)
Fatigue	2 (11%)	1 (7%)	0
Hyperglycemia	2 (11%)	1 (7%)	1 (10%)
Hypertension	1 (5%)	1 (7%)	0
Death	1 (5%)	0	0
Neuralgia	1 (5%)	0	0
Pollakiuria	1 (5%)	0	0
Depression	0	1 (7%)	0
Fracture	0	1 (7%)	1 (10%)
Hyperlipidemia	0	1 (7%)	1 (10%)

[a]All patients were analyzed including Patient 16. [b]Number was calculated by questionnaire on every visit. AE = adverse event; ED = erectile dysfunction.

SBMA.[51] The high tolerability of leuprorelin acetate was also supported by the low dropout rate in this trial.

An important limitation in this study is the trial duration. SBMA is a slowly progressive disease, with a disease duration of approximately 20 years.[7] Given that leuprorelin acetate did not suppress the decline in ALSFRS-R scores in our 48-week RCT, a long-term, placebo-controlled trial may be necessary to evaluate the efficacy of leuprorelin acetate on general motor function in SBMA. Based on this study, cricopharyngeal opening duration in VF appears to be a practical biomarker to evaluate therapy efficacy for SBMA in short-term trials.

In conclusion, the results of this study suggest that leuprorelin acetate administration suppresses nuclear accumulation, stabilization, or both of mutant AR, the causative protein of SBMA, and appears to inhibit functional deterioration of the patients. The results of this phase 2 trial support the start of large-scale clinical trials of androgen deprivation for SBMA.

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B-type natriuretic peptide and cardiovalvulopathy in Parkinson disease with dopamine agonist

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ABSTRACT

Objective: To elucidate the usefulness of plasma B-type natriuretic peptide (BNP) values for evaluating adverse effects of pergolide or cabergoline on cardiovalvulopathy in patients with Parkinson disease.

Methods: Twenty-five patients treated with pergolide or cabergoline (ergot group) and 25 patients never treated with ergot derivatives (non-ergot group) were enrolled. Plasma BNP values and detailed echocardiography were evaluated. Thirty age- and gender-matched controls were similarly evaluated.

Results: Patients with regurgitation more than grade 3 were more frequent in the ergot group than in the non-ergot group as well as control groups (24%, 0%, 3%, $p = 0.001$). Both composite regurgitation scores and plasma BNP values were significantly higher in the ergot group than in controls. In the ergot group, the cumulative dose correlated to both tenting area ($r = 0.57$, $p = 0.004$) and tenting distance ($r = 0.62$, $p = 0.001$). Furthermore, plasma BNP values were higher in patients with severe or multiple regurgitation groups ($p < 0.001$), and were correlated with composite regurgitation score ($r = 0.70$, $p < 0.001$). Multiple regression analyses revealed that BNP values were independently correlated with both composite regurgitation and left ventricular ejection fraction.

Conclusion: The combination of comprehensive echocardiography and plasma B-type natriuretic peptide levels elucidates the presence of cardiac damage in patients with Parkinson disease using ergot derivative dopamine agonists. ***Neurology*® 2009;72:621-626**

GLOSSARY

AR = aortic regurgitation; **BNP** = B-type natriuretic peptide; **MR** = mitral regurgitation; **PD** = Parkinson disease; **TR** = tricuspid regurgitation; **UPDRS** = Unified Parkinson's Disease Rating Scale.

Ergot derivative dopamine agonists including pergolide and cabergoline are some of the most effective drugs to treat parkinsonian symptoms and have the potential to reduce the motor complications observed in patients with Parkinson disease (PD) treated with L-dopa.[1] However, several reports have shown an association of ergot derivative dopamine agonists and cardiac multivalvular regurgitation.[2-18] In particular, high cumulative doses and long-term treatment with pergolide and cabergoline have been considered to be risk factors for increased valvulopathy in patients with PD. The US Food and Drug Administration public health advisory of March 29, 2007, cautions against abruptly stopping pergolide and is looking for ways to provide the drug to those people who cannot successfully switch to alternative treatments. On the contrary, over 60% of patients did not show valvulopathy, despite several years' exposure.[2] In Japan, similar to some European countries, pergolide and cabergoline are still used, and moderate doses of pergolide are associated with a low incidence of restrictive valvulopathy.[4,9]

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Although echocardiography is an essential tool to evaluate valvulopathy, a simple screening test that does not require specialized techniques would be beneficial for management of patients under various conditions in particular institutes without a department of cardiovascular medicine. B-type natriuretic peptide (BNP), which is secreted mainly from the heart and belongs to the natriuretic peptide family, is indicative of cardiac dysfunction in patients with not only heart failure and coronary artery disease but also valvular disorders.[19-21] Since plasma BNP values can be measured in serum by fully automated and commercially available assays with excellent test precision, it would be beneficial for monitoring the cardiac findings in patients with PD.

In this study, we investigated the usefulness of plasma BNP values for identifying and monitoring cardiac involvement in patients with PD treated with ergot derivative dopamine agonists.

METHODS The records of 121 patients with PD who attended the Department of Neurology, Nagoya University Hospital, and were nominated to our clinical cohort study at Nagoya area[22] during January to December 2006 were investigated. Of these, 34 patients with PD who fulfilled probable PD criteria according to the established diagnostic criteria[23] and were continuously taking ergot agonists (pergolide or cabergoline) but not non-ergot ones for a minimum of 1 year with or without levodopa were enrolled. Switching dopamine agonists between pergolide and cabergoline or combined use of pergolide and cabergoline often occurs in clinical practice. Although there are no controlled data for the comparison of two or more dopamine agonists to define equivalent dosages, several reports have been published on the clinical experience of experts.[24] In addition, the stimulus strength on 5-hydroxytryptamine 2B (5-HT_{2B}) receptors is similar between cabergoline and pergolide, in parallel with their molecular weights.[25,26] Thus, according to a previous report,[24] we calculated that 2 mg of pergolide is equal to 3 mg of cabergoline. Age- and sex-matched patients with PD who were never treated with ergot derivative dopamine agonists were also included. Six patients taking nonpermitted medication (anorectic or ergot alkaloid agents, Chinese herbs, anticancer or immune-suppressive drugs before enrollment), having a history of significant coronary heart disease, impaired function/dilatation of left/right ventricle, history of peripheral artery occlusive disease, and any clinically significant illnesses that may interfere with their capability to participate in the study were excluded. We also excluded three patients who were not treated in our institute because their history of taking dopamine agonists was found to be inaccurate. As a result, 25 patients treated with pergolide or cabergoline were enrolled in the ergot group. Thirteen patients were treated with pergolide or cabergoline. Five patients were only treated with pergolide and seven patients only with cabergoline. In addition, 25 patients never treated with ergot derivatives were also enrolled in the non-ergot group. Disease severity was assessed with the Unified Parkinson's Disease Rating Scale (UPDRS) and the Hoehn & Yahr stages. All patients showed normal renal function. Two of the ergot group and three of the non-ergot group patients had mild hypertension. Patients were interviewed with a structured questionnaire about the frequency of dyspnea, fatigue, leg edema, and palpitation, and were scored from 0 (no disability) to 4 (maximum). As for the controls, 30 age- and sex-matched normal volunteers who have no history of cardiac disorders and related conditions requiring medication were examined (age at examination: 67 ± 11 years; 16 women, 14 men). This study was approved by the ethics committee of Nagoya University Graduate School of Medicine. We obtained written informed consent from each participant before data collection.

All patients were assessed by an echocardiography GE VIVID 7 machine (GE Medical Systems, Milwaukee, WI) with two independent observers (A.N. and A.Y.) who were blinded to the clinical information. Mitral, aortic, and tricuspid valves were recorded from all possible views with the zoom function. In addition, a stethoscope examination was performed before echocardiography by A.N. and A.Y. Semiquantitative and quantitative measurements for quantification of regurgitant valvular diseases from the continuous wave, pulsed wave, and color Doppler examinations were assessed. Tenting distance and tenting area of the mitral valve were also evaluated as quantitative data.[7,8,10,15] We quantified regurgitant lesions by integration of all semiquantitative and quantitative measurements, and a final score was given as follows: absent, 0; trace, 1; mild, 2; moderate, 3; severe, 4.[27] A composite regurgitation score was calculated by adding the scores for aortic regurgitation (AR), mitral regurgitation (MR), and tricuspid regurgitation (TR).[7,15] The proportion of patients with any regurgitation grade from 3 to 4 was also assessed.[7] We derived the systolic pulmonary artery pressures from the TR jet, adding 10 mm Hg to the maximum gradient of the TR jet or 5 mm Hg if the vena cava inferior diameter was less than 10 mm with complete respiratory collapse and 15 mm Hg if the vena cava inferior was greater than 20 mm without respiratory variation. Left ventricular end-diastolic and end-systolic dimensions were measured, and the left ventricular ejection fraction was calculated by the Teicholz method. All patients with PD were investigated for both the diameter and flow of the hepatic vein and inferior vena cava using ultrasonography. In addition, a chest X-ray was also performed if necessary.

Blood for BNP quantification was collected in the fasting state in EDTA acid-treated tubes and placed on ice. After centrifugation at 2,500 rpm and 3°C, the plasma was stored at −80°C. BNP levels were measured directly with a specific immunoradiometric assay kit TOSOH AIA-PACK BNP (TOSOH Corp., Tokyo, Japan) including 30 age- and gender-matched controls.

Statistical analyses were performed using SPSS 15.0 for Windows (SPSS Inc.). Comparisons of age and disease duration between groups were performed using one-way analysis of variance followed by post hoc Bonferroni correction. Group comparisons of frequencies of valvular regurgitation were restricted to grades 3 and 4 and were performed using the Fisher exact test. The statistical threshold for post hoc comparisons between each treatment group vs the control group was set at $p < 0.017$ (0.05/3). The relationships between the cumulative dose of ergot derivative dopamine agonists and tenting area, tenting distance, composite regurgitation score, and BNP were analyzed using Pearson correlation test. Statistical significance was considered as $p < 0.05$.

RESULTS **Patient characteristics.** Patient characteristics were as follows: 22 men and 28 women; age at

examination, 66 ± 9 years; duration, 11.8 ± 9.6 years; mean Hoehn & Yahr stage 2.8. There were no significant differences between the ergot group and the non-ergot group in terms of Hoehn and Yahr staging (3.0 ± 0.9 vs 2.6 ± 0.9), duration of illness (12.9 ± 9.1 vs 10.6 ± 10.2 years), dosages of levodopa (494 ± 216 mg vs 481 ± 257 mg), age at examination (64.9 ± 9.2 vs 66.4 ± 7.7 years), and gender. Mean daily/cumulative dosage of pergolide was 1.1 ± 0.4/1,752 ± 1,512 mg and that of cabergoline was 3.1 ± 1.0/14,230 ± 2,566 mg. There were no patients who required a surgical operation during the course of this study. The frequency of leg edema, dyspnea, palpitation, and fatigue were not significantly different between the ergot group and the non-ergot group. All patients demonstrated more than 50% ejection fraction and did not show heart failure fulfilling the criteria of the Framingham study.[28,29]

Echocardiographic findings and plasma BNP levels. With respect to regurgitation, frequencies of equal to or greater than grade 3 regurgitation were only observed in the ergot group (12%, aortic valve; 12%, mitral valve; and 8%, tricuspid valve), except for one subject in the control group. The frequency of any grade 3 to 4 regurgitation was significant between the ergot group and non-ergot group as well as the ergot group and control group (ergot group vs non-ergot group; $p < 0.001$, ergot group vs control group; $p < 0.001$). Composite regurgitation scores in the ergot group were higher than in the control group ($p < 0.001$), but there were no differences between the ergot group and non-ergot group as well as between the non-ergot group and control group. Differences of tenting area and tenting distance between the ergot group and non-ergot group were slight (tenting area; ergot group, 1.26 ± 0.42, non-ergot group, 1.05 ± 0.21, $p = 0.04$, tenting distance; ergot group, 7.53 ± 2.57, non-ergot group, 6.10 ± 1.65, $p = 0.09$).

The plasma BNP levels as well as the composite regurgitation score were elevated in the ergot group vs the control group ($p = 0.004$, $p < 0.001$) (table). The BNP levels and composite regurgitation score in the ergot group showed a tendency to be increased as compared to those in the non-ergot group but did not show a significant difference. The BNP levels and composite regurgitation score in the non-ergot group were slightly elevated compared to control, although there was no significant difference.

Relationship between cumulative dose of ergot derivative dopamine agonists and echocardiographic findings. The cumulative dose of ergot derivative dopamine agonists was related to tenting distance ($r = 0.62$, $p = 0.001$) as well as to tenting area ($r = 0.57$, $p = 0.004$) but did not show any relationship with the composite regurgitation score ($r = 0.36$, $p = 0.08$). Within the high dose group (more than 4,000 mg), 33.3% of patients showed grade 3 to 4 regurgitation, while 15.4% of the low dose group (less than 4,000 mg) exhibited similar grades.

Plasma BNP levels in patients with severe valvulopathy. Plasma BNP values were higher in the ergot patient group with grade 3 to 4 regurgitation, which were seen only in the ergot group, than in those without such a high grade of regurgitation in the ergot group as well those in the non-ergot group (65.3 ± 47.8 pg/mL vs 24.7 ± 17.1 pg/mL vs 21.1 ± 15.4 pg/mL, $p < 0.001$). Patients with multiple regurgitation equal to or greater than grade 2 also had higher BNP values than those without (57.8 ± 46.1 vs 22.5 ± 11.9 vs 21.1 ± 15.4 pg/mL, $p < 0.001$).

According to receiver operating characteristic curve analyses to determine the adequate values for discriminating patients with severe regurgitation from those without, the most appropriate cutoff level of plasma BNP was 39.6 pg/mL, which showed 67.4% sensitivity and 84.4% specificity. In the ergot group, the positive predictive value was 66.7% and the negative predictive value was 89.4% if the plasma BNP level of 39.6 pg/mL was determined as the cutoff value.

Relationship between BNP and echocardiographic findings. The BNP levels showed a correlation to the composite regurgitation scores ($r = 0.70$, $p < 0.001$, figure) and a correlation to the left ventricular ejection fraction ($r = -0.42$, $p < 0.04$) but not age at examination, motor examination section (part III) of the UPDRS, and disease duration. Multiple regression analyses demonstrated that BNP values were independently correlated with composite regurgitation scores ($t = 4.08$, $p = 0.001$) and the left ventricular ejection fraction ($t = -2.07$, $p = 0.045$, $R^2 = 0.60$).

DISCUSSION We demonstrated that a significant elevation of plasma BNP values was observed in the ergot group vs in control groups. In particular, the BNP values were significantly elevated in the ergot group with more severe or multiple regurgitation than those with no to mild regurgitation and those in the non-ergot group. Furthermore, composite scores of regurgitation were well correlated with BNP values. Serum BNP is elevated in patients with valvular disorders due to ventricular pressure and volume load,[20,21] and this may be one reason why plasma BNP values increased in the ergot group. More recently, animal models have demonstrated that left ventricular cardiomyocytes were hypertrophic in both serotonin and pergolide-treated animals com-

Table Valvular abnormalities and plasma BNP values in the ergot group and non-ergot group

Grade of regurgitation, no. (%) of patients	Ergot group (n = 25)	Non-ergot group (n = 25)	Control (n = 30)
Aortic regurgitation			
0 to 1	13 (60)	22 (88)	27 (90)
2	7 (28)	3 (12)	3 (10)
3	2 (8)	0 (0)	0 (0)
4	1 (4)	0 (0)	0 (0)
Mitral regurgitation			
0 to 1	13 (60)	18 (72)	26 (87)
2	7 (28)	7 (28)	3 (10)
3	3 (12)	0 (0)	1 (3)
4	0 (0)	0 (0)	0 (0)
Tricuspid regurgitation			
0 to 1	19 (76)	21 (84)	25 (83)
2	4 (16)	4 (16)	5 (17)
3	1 (4)	0 (0)	0 (0)
4	1 (4)	0 (0)	0 (0)
Any grade from 3 to 4 regurgitation, mean (SD)	6 (24)*	0 (0)	1 (3)
Composite regurgitation score	3.30 (2.31)†	2.39 (1.29)	1.73 (1.83)
BNP (pg/mL)	33.6 (31.8)‡	21.1 (15.4)	14.2 (8.3)

Composite regurgitation score is the sum of mitral, aortic, and tricuspid regurgitation scores.

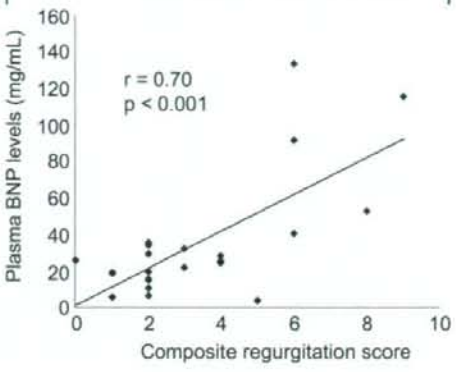
* The frequency of any grade 3 to 4 regurgitation was statistically significant between the ergot group and the non-ergot group ($p < 0.0001$) as well the ergot group and the control group ($p < 0.001$).

† Composite regurgitation score in the ergot group was significantly higher than that in the control group ($p < 0.001$). Composite regurgitation score of the ergot group tended to be increased when compared to the non-ergot group, but was not significantly different. Patients with grade 3 to 4 regurgitation were seen only in the ergot group.

‡ The plasma BNP level was significantly elevated in the ergot group vs in the control group ($p = 0.004$). The plasma BNP level of the ergot group tended to be increased when compared to the non-ergot group, but was not significantly different. The plasma BNP of patients with grade 3 to 4 regurgitation seen only in the ergot group was significantly elevated.

BNP = B-type natriuretic peptide.

Figure Correlation between composite regurgitation scores and serum B-type natriuretic peptide (BNP) values



Composite regurgitation scores were correlated with BNP values ($r = 0.70$, $p < 0.001$). Composite regurgitation score was calculated by adding the score of aortic regurgitation, mitral regurgitation, and tricuspid regurgitation.

pared with placebo-treated animals, and macroscopically, left ventricular cavities were more dilated in both the serotonin and pergolide groups.[30] Thus, the second possible explanation is that direct toxic effects on the cardiomyocytes may have an influence on increased plasma BNP values. Since ventricular involvement in patients with PD using ergot derivative dopamine agonists has not been fully assessed, further prospective and pathologic studies will be needed to clarify this issue.

BNP is expected to detect preclinical structural and functional myocardial alterations not detectable by current techniques. Thus, BNP testing for structural heart disease screening in community-based populations is useful for cohorts with a high prevalence of heart disease.[31,32] However, age, renal dysfunction, and fluid overload can also contribute to elevated BNP concentrations.[33] Furthermore, elderly hypertensive subjects with orthostatic systolic blood pressure decrease also show significantly higher BNP values than in the control group, suggesting greater cardiac burden although the influence of orthostatic hypotension on BNP is not well known.[34] In this study, no patients exhibited symptomatic orthostatic hypotension but sympathetic dysfunction in PD might result in a slight elevation of plasma BNP levels in the non-ergot group compared with controls. Since ergot derivative dopamine agonists can exacerbate not only cardiac fibrosis but also renal dysfunction and orthostatic hypotension, measurement of plasma BNP values may be beneficial to detect and prevent the worsening of these clinical conditions by means of administration of such dopamine agonists.

This study showed that plasma BNP values were significantly higher in the ergot group than in controls, while the plasma BNP values showed a tendency to be elevated in the ergot group vs the non-ergot group, but did not show a significant difference. In this study, over three-quarters of patients in the ergot group did not develop significant valvular regurgitation. Such a low occurrence of severe valvulopathy may be a consequence of the lower dose of pergolide and cabergoline prescribed to our Japanese patients when compared to Western countries.[2,9] However, six patients of the ergot group with grade 3 to 4 regurgitation clearly showed a significant elevation of plasma BNP values compared to those of the non-ergot group. These results demonstrate that plasma BNP values can be used as a marker for patients who have reached a significant degree of heart valve involvement prior to heart failure.

This study also showed that the composite regurgitation score in the non-ergot group showed a slight elevation when compared to controls. We cannot rule out the possibility that other drugs including L-dopa or sympathetic dysfunction have an influence on the increase of regurgitation in the non-ergot group, but no patients with grade 3 to 4 regurgitation were observed in this group. According to a recent review, a considerably large proportion of patients do not develop valvulopathy, despite several years' exposure to high doses of pergolide, suggesting the presence of patients with a low susceptibility to pergolide.[2] Furthermore, the low dose of pergolide used in Japan can be associated with the low frequency of severe valvulopathy in our patients treated with ergot as mentioned above.[2,9] The striking point here is, as mentioned above, patients with a grade 3 to 4 composite score were present in the ergot group but not in the non-ergot group.

Both pergolide and cabergoline are potent agonists of not only dopamine but also the 5-HT_{2B} receptor. It is supposed that stimulation of 5-HT_{2B}, which is expressed in heart valves, induces prolonged activation of fibroblast mitogenesis resulting in valvular fibroplasia.[35,36] Thus, high dose and long-term ergot derivative administration is thought to be a risk factor for valvulopathy in patients with PD.[2-18] The significant association of the cumulative dose of ergot derivatives and mitral valve tenting area/distance, which have been proposed as restrictive changes due to valvular fibroplasia, was observed.[7,8,10,15] However, these significant adverse events did not occur in all ergot patients, including those administered high cumulative doses as previously reported,[2] suggesting that patients who receive benefit from ergot-derived dopamine agonists without valvulopathy will exist at a constant rate under careful follow-up.

In Germany, if any abnormalities are seen on echocardiography, non-ergot dopamine agonists are recommended.[37] Although there has been no report concerning plasma BNP values in patients with PD, our results support the view that plasma BNP levels will be a beneficial marker for monitoring cardiac fibrosis due to ergot derivative dopamine agonists. Measurement of plasma BNP levels is quicker, more accessible, and cheaper than echocardiography and may contribute to the assessment of not only the development of valvulopathy and myocardium damage but also several other important factors deteriorated by ergot derivative dopamine agonists in patients with PD. In addition, plasma BNP values can predict the prognosis of patients with chronic heart failure[38] and mitral regurgitation.[39] Echocardiography is effective and able to identify valvulopathy as a cause of incipient or present right heart failure, and it is a satisfactory screen for valvulopathy itself, but BNP is a suitable marker for the relevant forms of cardiac dysfunction. The combination of comprehensive echocardiography and plasma BNP levels will complementarily elucidate the presence of cardiac damage in patients with PD using ergot derivative dopamine agonists.

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Age at onset influences on wide-ranged clinical features of sporadic amyotrophic lateral sclerosis

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ABSTRACT

Purpose: To profile the detailed clinical features of sporadic amyotrophic lateral sclerosis (ALS) on large-scale samples in Japan.
Methods: We assessed the clinical features of sporadic ALS patients in Japan, based on the nationwide registration system of the Ministry of Health, Labor and Welfare of Japan. We described 3428 new cases registered cases between 2003 and 2006 to analyze initial symptoms and related clinical features, 4202 cases registered in the single year of 2005 to describe the cross-sectional overview of the ALS patients, and a total of 2128 cases with tracheostomy positive pressure ventilation (TPPV) from all of the registration data from 2003 to 2006 to describe the features of ALS patients with TPPV.
Results: The patients with an older age at onset progressed more rapidly to the TPPV stage than those with a younger age at onset. The subpopulation of patients with long-standing TPPV showed ophthalmoplegia, while its appearance rate was less in the patients with an older age at onset than in those with a younger age at onset. Furthermore, age at onset strongly influenced the frequency of initial symptoms: dysarthria, dysphagia, neck weakness and respiratory disturbance were more frequent in patients with an older age at onset, while upper or lower limb weakness was observed more frequently in patients with a younger age at onset. In addition, those initial symptoms were still the most prominent at the follow-up stage, suggesting that the initial symptoms determine the major clinical features even in advanced illness.
Conclusions: Our present study demonstrated that symptomatic features of ALS are strongly influenced by the age at onset by the large scale of samples.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is one of the most devastating neurodegenerative diseases affecting upper and lower motor neurons preferentially, and shows progressive muscle wasting of the limb, bulbar and respiratory musculatures. Almost half of ALS patients expire within three years of onset, primarily due to respiratory failure [1–6]. Approximately 5–10% of ALS patients show a familial trait, while more than 90% of the patients are sporadic, and the causal mechanism of the motor neuron degeneration is largely unknown. Although many clinical trials of potential therapeutic agents for the treatment of sporadic ALS have been performed [7], effective therapeutics against motor neuron degeneration in ALS except for riluzole [8,9] have not been developed. The clinical features of ALS have been established for the most part. However, many aspects of symptomatic manifestations such as the influence of age at onset on clinical features, the frequency of rare symptoms and many other symptomatic details have not been well characterized, particularly

Abbreviations: ALS, amyotrophic lateral sclerosis; TPPV, tracheostomy positive pressure ventilation.

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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

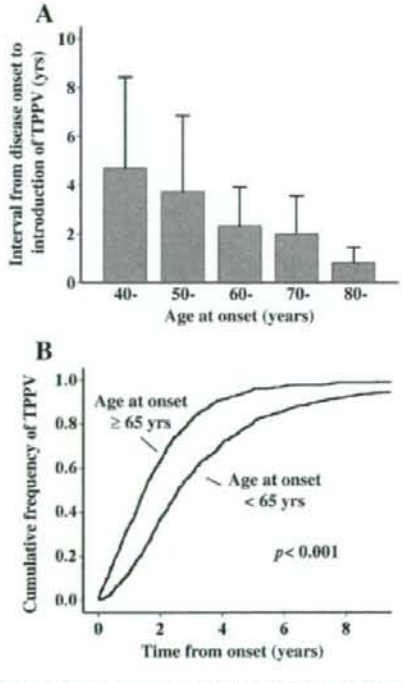

Fig. 1. Relationship between age at onset and introduction of tube feeding and TPPV. Interval from disease onset to introduction of TPPV (A) is shown. An older age at onset strongly correlates to shorter intervals from onset to TPPV. Cumulative frequencies of patients with TPPV in the patient population 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.

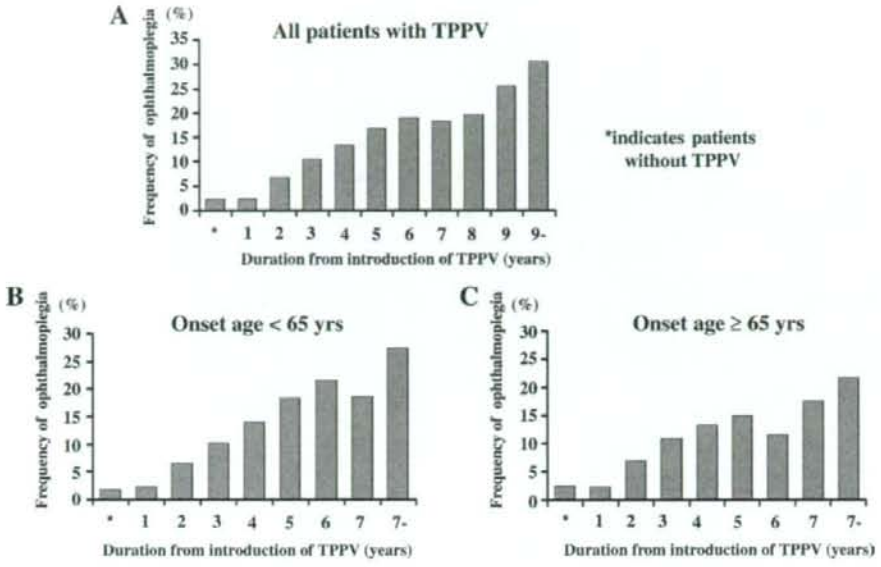

Fig. 2. Frequency of ophthalmoplegia in patients under TPPV, in terms of duration of TPPV and the influence of onset age on its appearance. Ophthalmoplegia rarely occurs in patients without TPPV (*), while its occurrence gradually increases with advanced duration of TPPV (A). Following 9 years of TPPV, 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

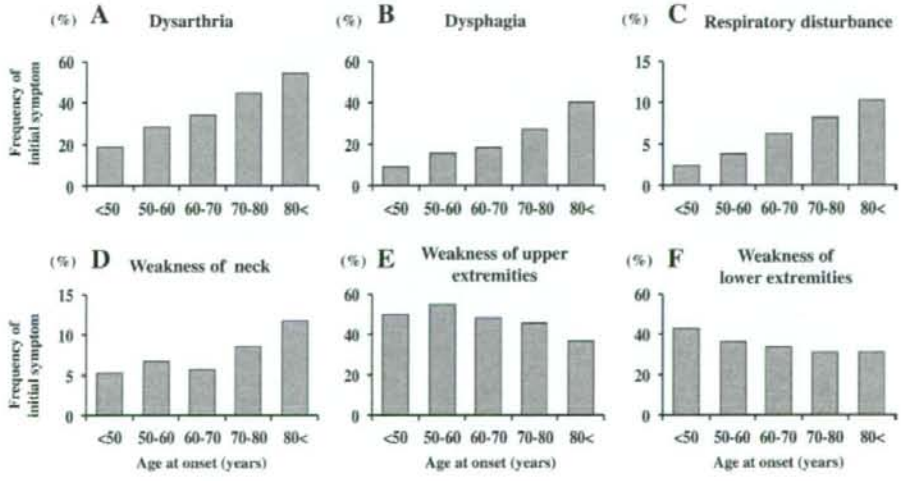

Fig. 3. Age at onset and frequency of initial symptoms. Dysarthria (A), dysphagia (B), respiratory disturbance (C) and weakness of neck (D) are increased in frequency as an initial symptom as the age at onset increases. In contrast, weakness of the upper extremities (E) or lower extremities (F) decreased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

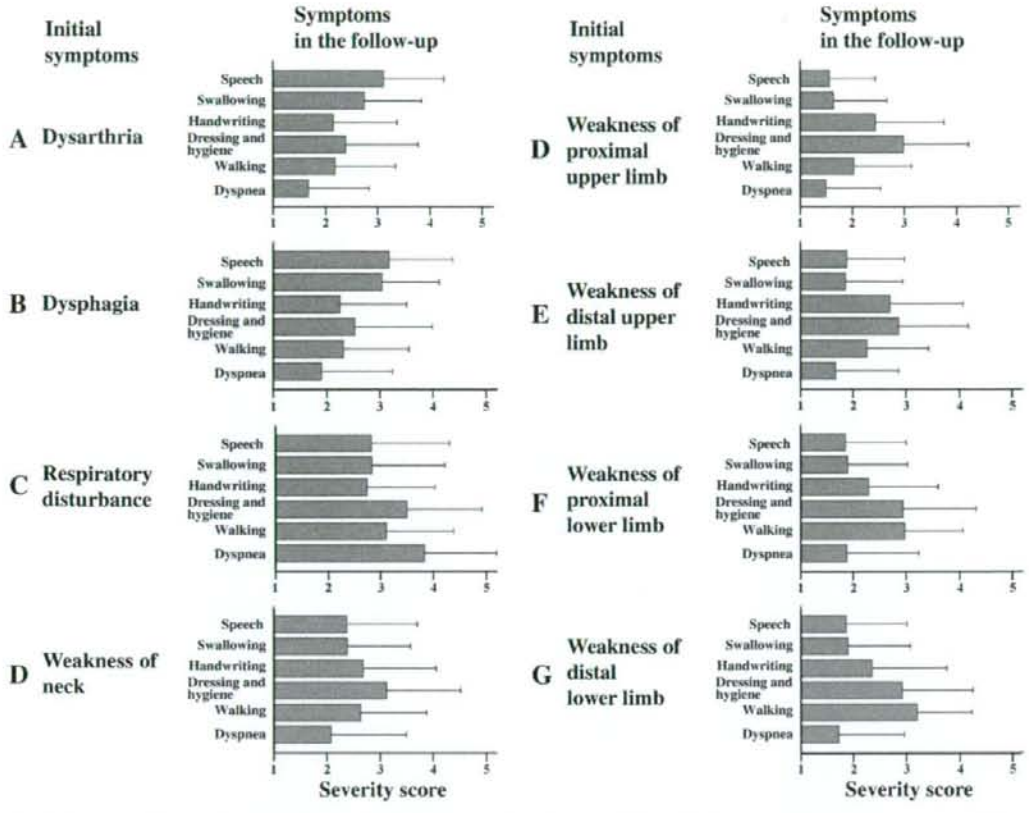

Fig. 4. Relationship between initial symptoms and symptoms at the follow-up stage. Severity scores of Speech, Swallowing, Handwriting, Dressing and Hygiene, Walking and Dyspnea are shown as subscales of ALSFRS-R. The score of "5" represents the most severe state, and "1" represents the absence of the 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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