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RESEARCH Open Access

A 3-year cohort study of the natural history of spinocerebellar ataxia type 6 in Japan

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Abstract

Background: Only a few prospective studies have determined which clinical symptoms and factors are associated with the disease severity of spinocerebellar ataxia type 6 (SCA6). A multicenter longitudinal cohort study was conducted to clarify both the natural history of SCA6 in Japan and the factors influencing disease progression.

Methods: Patients were consecutively recruited between 2007 and 2008. Scores from the Scale for the Assessment and Rating of Ataxia (SARA) and Barthel Index (BI) were collected prospectively each year. Additionally, data from the Japan intractable diseases research (IDR) registry were collected both retrospectively, from 2003 to 2006, and prospectively, from 2007 to 2010. As a result, we were able to collect 3 years of retrospective data and 4 years of prospective data during the course of 3 yearly visits.

Results: Forty-six patients were registered. The follow-up rate of the third year was 93%. The SARA scores worsened significantly each year. Over 3 years, the decline of the SARA scores was 1.33 ± 1.40 points/year. The results of multivariate analysis of the decline of the SARA score were not significant. The IDR scores correlated well with the SARA and BI scores. Kaplan-Meier curves of 7 years of data from the IDR registry illustrated the correlation between the ability to walk and the time course of the disease.

Conclusions: Information regarding the progression of ataxia and the decline in the activities of daily living (ADL) in patients with SCA6 was obtained by a 3-year cohort study and a 7-year IDR study. The decline of the SARA score of patients with SCA6 was 1.33 ± 1.40 points/year. The results elucidate the natural history of SCA6, factors influencing disease severity, and utility of data from the IDR registry of Japan.

Keywords: Barthel Index, CAG repeat, International Cooperative Ataxia Rating Scale, Intractable diseases research, Scale for the Assessment and Rating of Ataxia, Spinocerebellar ataxia

Background

The spinocerebellar ataxias (SCAs) are neurodegenerative diseases characterized by oculomotor disturbances, dysarthria, limb and truncal ataxia, gait disturbances, and additional variable symptoms [1]. In the 1990's, genetic mapping studies in patients with autosomal dominant cerebellar ataxias (ADCAs) identified 7 polyglutamine diseases: SCA type 1 [SCA1], SCA2, SCA3, SCA6, SCA7, SCA17, and dentatorubral-pallidoluysian atrophy [DRPLA] [2].

Although numerous studies have described the clinical manifestations of the SCAs [2-4], very few prospective studies have examined which clinical symptoms and factors are associated with disease severity. Knowledge of the natural history of the SCAs is required to counsel patients and to design interventional trials. One cohort study of SCA, the European EUROSCA natural history study, was a multicenter longitudinal study that included 526 patients with SCA1, SCA2, SCA3, and SCA6 [5-8]. A 2-year follow-up study of EUROSCA used several scales, including the Scale for the Assessment and Rating of Ataxia (SARA), to describe disease progression and identify factors that specifically affected this process [8]. Recently, Ashizawa et al. reported the results of a

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prospective study of the natural history of the SCAs in the United States [9]. We designed a multicenter longitudinal cohort study of the natural history of SCA6 in Japan. Our study used the SARA and the Barthel Index (BI). SCA6 was selected because of the high prevalence of patients with this disease in Japan.

In Japan, the Ministry of Health, Labour, and Welfare has established a national registry system for the survey of 'intractable diseases' such as the SCAs [10,11]. The Ministry is conducting a project that subsidizes the medical expenses of patients with an 'intractable disease'. This project also supports research activities on these diseases because effective treatments have not yet been established and the affected patients have considerable disabilities. Each year, patients with an 'intractable disease' submit a 'clinical inquiry sheet,' which is completed by their physician, to the IDR. Since 2003, the inquiry sheet for patients with an SCA has included 5 items from the International Cooperative Ataxia Rating Scale (ICARS) and 6 items from the BI [12,13]. The purposes of the IDR registry are to provide financial support for patients and investigate the patients' clinical states; however, data from the present IDR registry have not yet been utilized for longitudinal natural history studies.

The primary aims of this study were to longitudinally and quantitatively investigate the clinical severity, disease progression, and natural history of SCA6, to identify factors that specifically affected disease progression in a 3-year prospective study in Japan, and to compare the results of this study with those of previous studies. The secondary aim was to examine the reliability and utility of the data collected by the Ministry of Health, Labour, and Welfare's national IDR registry.

Methods

Patient registration

The present study was performed at 8 centers (Hokkaido University, Niigata University, Chiba University, National Hospital Organization Chiba-East-Hospital, Tokyo Metropolitan Neurological Hospital, Shinsyu University, Nagoya University, and Tottori University) belonging to the Research Committee for Ataxic Disease. This committee is part of the Ministry of Health, Labour, and Welfare of Japan. The written informed consent form, which was approved by the institutional review board of all centers and by the Ethics Committee of the Tottori University Hospital, was signed by all study participants. Patients with SCA6 who were being treated at any of the 8 centers were consecutively recruited between 2007 and 2008. A linkable, anonymizing registration system was used to register all patients.

The patients did not provide DNA samples for this study and therefore diagnoses based on DNA analyses were made in accordance with the protocols being used at each center. Information regarding the CAG repeat length of the expanded allele of the alpha 1A P/Q type voltage-dependent calcium channel gene (*CACNA1A*) of each patient was obtained from the medical records of the respective center.

Procedures of the 3-year prospective study

Registration and follow-up evaluations were performed from April to July during the annual registration period of the Japan IDR registry; therefore, follow-up investigations were performed at the same time each year. As part of the prospective study, all patients were assessed with the SARA and the BI each year. The SARA was used to assess the degree of ataxia. The SARA consists of 8 items: gait, stance, sitting, speech, finger chase, nose-to-finger test, fast alternating hand movements, and heel-to-shin slide. Each item has its own subscore. The SARA grades ataxia on a scale of 0 to 40, with 0 indicating the absence of ataxia and 40 indicating the most severe degree of ataxia. The Japanese SARA is well validated and can be administered quickly [14,15]. The BI was used to assess how well a patient performs the activities of daily living (ADL). The BI grades activity on a scale of 0 to 100, with 0 indicating that a person cannot care for him/herself and 100 indicating that a person can care for him/herself. All investigators were board-certified neurologists and were experienced in the use of the applied scales.

Procedures of the 7-year IDR registry study

The IDR inquiry sheet for patients with an SCA includes 5 items from the ICARS (walking, standing with eyes open, body sway with feet together and eyes closed, knee-tibia test of the worse foot, and finger-to-nose test of the worse hand [decomposition and dysmetria]) and 6 items from the BI (feeding, bathing, grooming, dressing, mobility, and stairs) [11-13]. The total number of points obtained from the IDR inquiry sheet for the 5 items from the ICARS and the 6 items from the BI was referred to as the IDR-ICARS and IDR-BI scores, respectively. The IDR-ICARS grades ataxia on a scale of 0 to 26, with 0 indicating the least impaired condition. The IDR-BI grades activity on a scale of 0 to 55, with 0 indicating the most impaired condition. The 5 items of the IDR-ICARS were selected because these subscores correlated well with disease duration in Japanese patients with cerebellar ataxia [10].

The IDR inquiry sheet was last modified in 2003. For the retrospective portion of the IDR registry study, we collected the IDR inquiry sheets from 2003 onwards. The inquiry sheets were collected from the patients' medical records, starting in the year they registered for the study. IDR data collection continued prospectively

each year. The utility of the IDR registry was evaluated by analyzing a total of 7 years' worth of data.

Data analysis

The differences between the scores obtained at registration and those obtained at each year's evaluation were referred to as the Δ scores, and the differences between the scores obtained at registration and the scores obtained at the last evaluation were referred to as the total Δ scores. The total Δ/year was calculated by dividing the total Δ score by the number of follow-up years.

Statistical analyses were performed with IBM SPSS Statistics software version 19 (SPSS Inc., Chicago, IL). The test results were considered significant at the .05 level. The Mann–Whitney test was used to compare the clinical characteristics of the male and female patients. Correlations between clinical scores and covariates were tested by using the Pearson correlation test. Data for disease progression were analyzed by using the Friedman test followed by post hoc Wilcoxon signed rank tests. The rate at which patients became wheelchair dependent was calculated from the data of the 7-year IDR registry study by using the Kaplan-Meier method. The CAG repeat length of the normal *CACNA1A* allele of each patient could not be collected in this study; therefore, we analyzed the repeat lengths of the expanded alleles, with the exception of the repeat lengths of the 3 patients who are homozygous for the expanded allele. For the cross-sectional study, an analysis of covariance at registration was performed with the SARA score as the dependent variable and sex, age at onset, disease duration, and CAG repeat length of the expanded *CACNA1A* allele as independent variables. For the prospective study, an analysis of covariance was performed with the total ΔSARA/year as the dependent variable and sex, age at onset, disease duration, CAG repeat length of the expanded *CACNA1A* allele, and SARA score at registration as independent variables. Age at registration was not included in the model, as age at registration was recorded as the sum of age at onset and disease duration. The test results were considered significant at the .01 level for the multivariate analysis.

Results

Patient characteristics

The study population consisted of 46 patients with SCA6 who belonged to 44 families. The SARA and IDR-ICARS scores of female patients were significantly lower than those of male patients. Although the age at onset, age at registration, and disease duration tended to be lower in female patients than in male patients, the differences were not statistically significant. Similarly, although the BI and IDR-BI scores of female patients tended to be higher than those of male patients, the differences were not statistically significant (Table 1).

Table 1 Demographic, genetic, and clinical characteristics of the study population

	All patients	Male patients	Female patients
No.	46	23	23
Age at onset, y	48.0 ± 9.3 (31–66)	48.8 ± 10.0	47.2 ± 8.6
Age at registration, y	63.0 ± 9.6 (41–78)	64.4 ± 10.0	61.5 ± 9.2
Disease duration, y	15.0 ± 8.0 (3–40)	15.6 ± 7.5	14.3 ± 8.7
SARA score, points (Range, 0–40)	15.9 ± 7.1 (4–33)	18.2 ± 6.2	13.6 ± 7.3*
BI score, points (Range, 0–100)	77.4 ± 22.4 (15–100)	72.2 ± 23.0	82.6 ± 20.9
IDR-ICARS score, points (Range, 0–26)	14.8 ± 6.0 (5–26)	16.6 ± 5.6	13.1 ± 6.1*
IDR-BI score, points (Range, 0–55)	36.7 ± 15.1 (5–55)	33.5 ± 16.1	40.0 ± 13.7
CAG repeat length of the expanded *CACNA1A* allele[a]	23.2 ± 1.4 (21–27)	23.2 ± 1.3	23.3 ± 1.6

Where applicable, the values are given as the mean ± standard deviation (range).
Abbreviations: BI = Barthel Index; *CACNA1A* = alpha 1A P/Q type voltage-dependent calcium channel gene; CAG = cytosine-adenine-guanine; ICARS = International Cooperative Ataxia Rating Scale; IDR = Intractable Diseases Research; SARA = Scale for the Assessment and Rating of Ataxia.
IDR-BI = total points of 6 items from the BI assessed by the IDR registry.
IDR-ICARS = total points of 5 items from the ICARS assessed by the IDR registry.
[a]Three patients are homozygous for the expanded allele (repeat lengths: 20, 22, and 24). The mean ± standard deviation was applied for 43 patients heterozygous for the expanded allele (22 male and 21 female patients).
*The scores of female patients were significantly lower than those of male patients ($P < .05$, Mann–Whitney test).

Correlations between clinical scores and factors at registration

A patient's age at the time of registration and the duration of his/her disease were correlated with clinical scores; however, a patient's age at the time of disease onset and the CAG repeat length of the expanded *CACNA1A* allele were not correlated with those scores (Table 2).

The patients' IDR-ICARS and IDR-BI scores correlated well with their SARA and BI scores, respectively (Figure 1A, B). The correlation coefficients of the SARA and IDR-ICARS scores and the BI and IDR-BI scores were 0.89 and 0.93, respectively ($P < .001$). The BI scores were inversely correlated with the SARA scores ($R = -0.83$, $P < .001$). Patients with a SARA score of less than 10 points maintained a high BI score. Conversely, among patients with a SARA score of more than 10 points, those with a higher SARA score had a lower BI score (Figure 1C).

The results of multivariate analysis of the patients' SARA scores at the time of registration are presented in Table 3. An analysis of covariance with the SARA score as the dependent variable and clinical factors as independent variables produced multivariate models that explained 39.5% of the variance of the SARA scores. The SARA scores were influenced by sex, age at onset,

Table 2 Correlations between the SARA, IDR-ICARS, BI, and IDR-BI scores and patients' demographics

	SARA	IDR-ICARS	BI	IDR-BI
Age at onset	NS	NS	NS	NS
Age at registration	0.36[b]	0.43[c]	−0.45[c]	−0.57[c]
Disease duration	0.35[b]	0.45[c]	−0.35[b]	−0.44[c]
CAG repeat length of the expanded *CACNA1A* allele[a]	NS	NS	NS	NS

Correlation coefficients are presented.
Abbreviations: BI = Barthel Index; *CACNA1A* = the alpha 1A P/Q type voltage-dependent calcium channel gene; CAG = cytosine-adenine-guanine; ICARS = International Cooperative Ataxia Rating Scale; IDR = Intractable Diseases Research; NS = not significant; SARA = Scale for the Assessment and Rating of Ataxia.
IDR-BI = total points of 6 items from the BI assessed by the IDR registry.
IDR-ICARS = total points of 5 items from the ICARS assessed by the IDR registry.
[a]Data on the CAG repeat length were analyzed for 43 patients heterozygous for the expanded allele.
[b]$P < .05$ (statistical analysis conducted with the Pearson correlation test).
[c]$P < .01$ (statistical analysis conducted with the Pearson correlation test).

disease duration, and CAG repeat length of the expanded *CACNA1A* allele.

Findings of the 3-year prospective study

The data obtained from the prospective study were used to obtain information on disease progression. This information is presented in Table 4 (5 right most columns). During the 3-year follow-up period, 2 patients died and 1 patient dropped out. The causes of the 2 deaths were suffocation and aplastic anemia. One untraceable patient could not continue to visit because of his disability. The follow-up rate of the third year was 93%. The total number of evaluations for the prospective study was 177. The SARA scores worsened significantly each year. The ΔSARA/year at the 1-, 2-, and 3-year follow-up evaluations was 1.35 ± 1.70 (mean ± SD), 1.39 ± 1.39, and 1.24 ± 1.06 points/year, respectively. The total ΔSARA/year was 1.33 ± 1.40 points/year. The ΔSARA/year was 1.08 ± 0.92 points/year for male patients and 1.56 ± 1.71 points/year for female patients. The difference of the ΔSARA/year between genders was not significant. Each year, the SARA scores of patients who scored between 0 and 24.5 points changed by similar amounts (ΔSARA/year = 1.48 ± 1.86 points/y), but the amount of change was smaller for patients with scores of more than 25 points (ΔSARA/year = 0.48 ± 1.42 points/y).

Among the subscores of the SARA scale, the subscores for gait (Δ/year = 0.27 points/y), stance (Δ/year = 0.22 points/y), and fast alternating hand movements (Δ/year = 0.24 points/y) were worse than the 5 other subscores (sitting: Δ/year = 0.10 points/y, speech: Δ/year = 0.16 points/y, finger chase: Δ/year = 0.06 points/y, nose-to-finger test: Δ/year = 0.10 points/y, heel-to-shin slide: Δ/year = 0.10 points/y) at the third year. The results of multivariate analysis for the decline in the SARA score were not significant.

Findings of the 7-year IDR registry study

The data from the 7-year IDR registry study were used to analyze disease progression. The results of this analysis are presented in Table 4 (last 4 rows). The ΔIDR-ICARS/year of the prospective portion of the study (absolute value 0.66 ± 1.28 points/y) was similar to that of the retrospective portion (absolute value 0.60 ± 1.59 points/y). The IDR-ICARS scores changed linearly in the retrospective and prospective portions of the study. The regression coefficient of the linear regression analysis was 0.63. Among the subscores of the IDR-ICARS scale, the subscore for walking (Δ/year = 0.23 points/y) was worse than the 4 other subscores (standing: Δ/year = 0.12 points/y, body sway: Δ/year = 0.11 points/y, knee-tibia test: Δ/year = 0.05 points/y, and finger-to-nose test: Δ/year = 0.07 points/y) at the third year.

Among the 46 patients with SCA6 who participated in this study, 9 already used a wheelchair when they filed their first IDR inquiry sheet. During the 7-year study period of the IDR registry, 12 of the remaining 37 patients became wheelchair dependent. Figure 2 panels A and B show the rates at which patients became wheelchair dependent. These rates are based on disease duration and age, respectively. All patients with a disease duration of less than 8 years and patients who were younger than 51 years could walk during the entire study. During the course of the study, patients with a disease duration of more than 9 years and patients who were older than 52 years gradually became wheelchair dependent. The medians of disease duration and age in patients who needed to use a wheelchair were 24.0 years (95% confidence interval [95% CI], 12.8 - 35.2 y) and 77.0 years (95% CI, 71.6 - 82.4 y), respectively. The 9 patients who were wheelchair dependent when they filed their first IDR inquiry sheet had a disease duration of more than 11 years and an IDR-ICARS score of more than 17 points, and all were older than 56 years.

Discussion

This 3-year prospective study elucidated the quantitative natural history of SCA6 in Japan. Although several previous studies have used a cross-sectional or retrospective design to describe the clinical characteristics of SCA6 [16-18], ours is the first to use SARA and BI scores to prospectively assess a Japanese cohort. Furthermore, this study provides an accurate natural history of SCA6 because of the high follow-up rate. Our success was possible because the study was performed in 8 centers belonging to the Research Committee for Ataxic Disease. This committee is part of the Ministry of Health, Labour, and Welfare of Japan. In addition, the follow-up

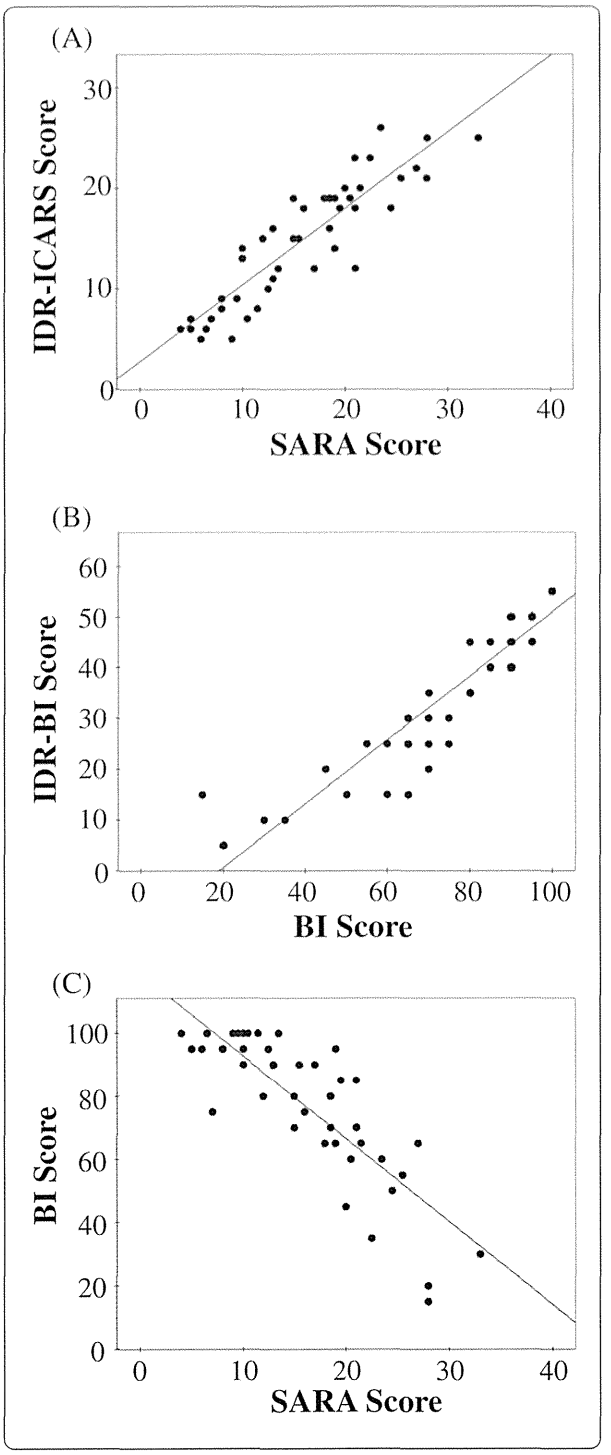

Figure 1 Relationships between the clinical scales used by the prospective study and by the IDR registry. (A) The relationship between the IDR-ICARS and SARA scales. The IDR-ICARS scores correlated well with the SARA scores (R = 0.892, $P < .001$, Pearson correlation test). **(B)** The relationship between the IDR-BI and BI scales. The IDR-BI scores correlated well with the BI scores (R = 0.928, $P < .001$, Pearson correlation test). **(C)** The relationship between the SARA and BI scales. The BI scores were inversely correlated with the SARA scores (R = −0.828, $P < .001$, Pearson correlation test). Abbreviations: BI = Barthel Index; ICARS = International Cooperative Ataxia Rating Scale; IDR = Intractable Diseases Research; SARA = Scale for the Assessment and Rating of Ataxia.

investigations were performed at the same time each year, during the annual registration period of the Japan IDR registry.

Regrettably, one disadvantage of this study is that DNA was not collected: only data for the CAG repeat length of the expanded allele of the *CACNA1A* gene was used in our analysis. We did not analyze the repeat lengths of the patients who are homozygous for the expanded *CACNA1A* allele. These data were collected from the patients' medical records. Given that the genetic data of this study were partially limited, we emphasized the clinical courses of the patients.

The correlation analysis of the patients' clinical scores and clinical factors revealed that a patient's age at the time of registration and the duration of his/her disease were correlated with the SARA score (Table 2). These results and the corresponding correlation coefficients are similar to those of previous reports [6]. Furthermore, the results of this study clarified the correlation between the SARA and BI scores (Figure 1C). Among the 46 participants in this study, female patients tended to be younger and have a shorter disease duration than male patients; however, the differences were not statistically significant. On the other hand, the SARA and IDR-ICARS scores of female patients were significantly less

Table 3 Results of multivariate analysis for the SARA score at registration

R^2	*P*	*Effect*	*Estimate*	*SE*	*β*	*t*	*P*
0.395	.001						
		Intercept	−45.558	21.830	n/a	−2.087	.044
		Sex	−5.669	1.775	−.415	−3.194	.003
		Age at onset	.338	.117	.451	2.877	.007
		Disease duration	.252	.122	.285	2.067	.046
		CAG repeat length of the expanded *CACNA1A* allele	2.132	.812	.421	2.627	.012

R^2 = Coefficient of determination; β = standard coefficient.
Abbreviations: *CACNA1A* = alpha 1A P/Q type voltage-dependent calcium channel gene; CAG = cytosine-adenine-guanine; n/a = not applicable; SARA = Scale for the Assessment and Rating of Ataxia; SE = standard error.

Table 4 Time course of disease progression based on the SARA, BI, IDR-ICARS and IDR-BI scores

Follow-up year	−4	−3	−2	−1	Registration	1	2	3	Total
N (follow-up rate%)	29	35	38	40	46(100)	44(96)	44(96)	43(93)	177*
Death, n					0	1	0	1	2
Withdrawal, n					0	1	0	0	1
SARA score, points					15.9 ± 7.1	16.8 ± 6.9[a]	18.2 ± 7.2[a]	19.1 ± 6.9[a]	17.5 ± 7.1
ΔSARA, points					0	1.35 ± 1.70	2.78 ± 2.78	3.73 ± 3.17	n/a
ΔSARA/year, points/y					0	1.35 ± 1.70	1.39 ± 1.39	1.24 ± 1.06	1.33 ± 1.40
BI score, points					77.4 ± 22.4	77.4 ± 22.3[b]	73.8 ± 23.7[b]	75.0 ± 23.2	75.9 ± 22.8
IDR-ICARS score, points	13.9 ± 5.9	13.7 ± 5.9	14.1 ± 6.0[c]	14.6 ± 5.7	14.8 ± 6.0	15.0 ± 5.4[b]	16.0 ± 5.7[b]	16.2 ± 5.6	14.9 ± 5.8
ΔIDR-ICARS, points	−2.28 ± 3.59	−2.06 ± 3.27	−1.29 ± 2.98	−0.50 ± 2.32	0	0.59 ± 1.65	1.59 ± 2.34	1.77 ± 2.79	n/a
ΔIDR-ICARS/year, points/y	−0.57 ± 0.90	−0.69 ± 1.09	−0.64 ± 1.49	−0.50 ± 2.32	0	0.59 ± 1.65	0.80 ± 1.17	0.59 ± 0.93	0.63 ± 1.45[d]
IDR-BI score, points	37.1 ± 14.0	38.6 ± 14.2	38.4 ± 13.8[c]	37.4 ± 14.1	36.7 ± 15.1	37.6 ± 15.0	34.9 ± 14.8[b]	35.8 ± 14.9	36.3 ± 14.9

Where applicable, the values are given as the mean ± standard deviation.
Comparisons of the IDR-ICARS, IDR-BI, SARA, and BI scores of each year were made by using the Friedman test followed by the Wilcoxon signed rank test as a post hoc test.
Abbreviations: BI = Barthel Index; ICARS = International Cooperative Ataxia Rating Scale; IDR = Intractable Diseases Research; n/a = not applicable; SARA = Scale for the Assessment and Rating of Ataxia.
IDR-BI = total points of 6 items from the BI assessed by the IDR registry.
IDR-ICARS = total points of 5 items from the ICARS assessed by the IDR registry.
ΔIDR-ICARS and ΔSARA = differences between the scores obtained at the follow-up evaluations and those obtained at registration for the IDR-ICARS and SARA, respectively.
[a]$P < .001$, versus the previous year for the prospective study.
[b]$P < .05$ versus the previous year for the prospective study.
[c]$P < .05$ versus the next year for the retrospective study.
[d]Total of the IDR-ICARS/year calculated with the absolute value of each ΔIDR-ICARS/year excluding that of the registration year.
*Total number of evaluations conducted during the prospective arm of the study.

than those of male patients. Thus, the patients' clinical backgrounds differed slightly between genders.

During the 3 years of this study, the SARA scores declined by 1.33 points/year, and the ΔSARA/year did not change significantly. This decline in the SARA score is greater than the 0.35 points/year decline that was observed for the first year of the EUROSCA study but it is similar to that observed for the second year (1.44 points/y) [8]. In a study conducted in the United States, the SARA score of patients with SCA6 declined by 0.87 ± 0.28 points/year [9]. In another study conducted in Asia, the SARA score of patients with SCA6 declined by 2.04 ± 0.76 points/year [19]. Although the reason for these differences is unclear, several explanations are possible. First, the backgrounds of the patients may be an important factor. Second, the dependence of the ΔSARA on the SARA score may be another reason why the scores differ: the ΔSARA/year for the patients with SARA scores of 25 points or more was less than that for the patients with SARA scores of less than 25 points. This finding may have important implications for the design of future clinical trials. Judging from the results of this study, we conclude that it may be appropriate to exclude patients with SARA scores of greater than 25 points. Third, the follow-up rate and the number of patients enrolled may also have been associated with the differences in the results of these studies.

The results of multivariate analysis for the decline in the SARA scores were not significant. The findings of a 2-year follow-up study by Jacobi et al. indicate that disease duration and CAG repeat length of the normal *CACNA1A* allele were independent factors associated with the decline in the SARA score in male patients with SCA6 [8]. We currently have no explanation for Jacobi et al's study because the genetic data and number of patients of this study were limited. Disease progression in patients with SCA6 may be affected by various factors including disease duration, age, and disease severity at the time of registration as well as by the CAG repeat length of the *CACNA1A* alleles. The 3-year prospective observation period of our study is longer than those of previous studies, and the length of the observation period is one of the most important factors for a study of the natural history of a disease. Furthermore, as the number of follow-up years increases, disease progression may become increasingly homogenous.

In addition to data generated by the 3-year prospective study, the 7-year IDR registry study provided long-term information on the natural history of SCA6. The amount of change of the IDR scores was small; therefore, the IDR inquiry sheet may not be suitable for use in clinical trials. However, the IDR inquiry sheet is simple and it can be completed in the setting of a typical daily clinic. Moreover, the IDR-ICARS and IDR-BI scores correlated

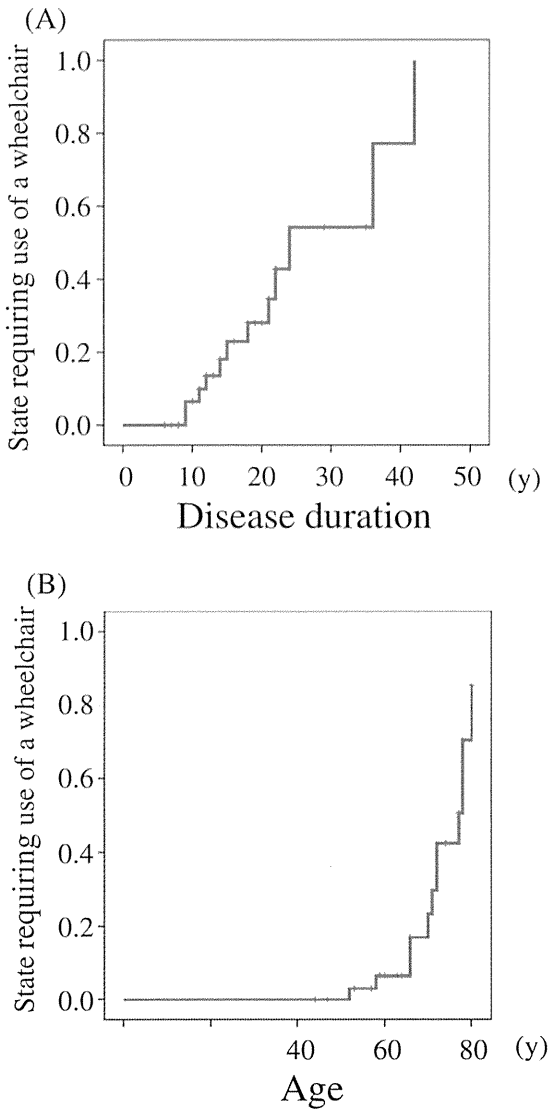

Figure 2 Utilization of ADL states as milestones indicating disease progression. Data from the 7-year IDR registry study was used to obtain information regarding the time that elapsed before patients became wheelchair dependent (**A**: Disease duration, **B**: Patient's age). Among 37 patients with SCA6, 12 became wheelchair dependent during the 7 years of the study. The Kaplan-Meier curves are shown. The medians (95% confidence interval) of disease duration and age for those patients who became wheelchair dependent were 24.0 years (12.8 – 35.2 y) and 77.0 years (71.6 – 82.4 y), respectively. Abbreviations: ADL = activities of daily living; SARA = Scale for the Assessment and Rating of Ataxia; SCA6 = spinocerebellar ataxia type 6.

well with the SARA and BI scores, respectively. Therefore, the IDR inquiry sheet could be useful for an uncomplicated follow-up study. The 7-year IDR registry study allowed us to monitor the long-term progression of ataxia, as indicated by the changes in the IDR-ICARS scores and the decline in the ADL, which are measured by the IDR-BI. Kaplan-Meier curves described the correlation between the ability to walk and the time course of the disease. A long-term period of observation is indispensable for a thorough understanding of the natural history of the SCAs.

Conclusions

This study clarified the natural history of SCA6 by performing a 3-year prospective study and by analyzing 7 years' worth of data from the IDR registry. The decline of the SARA score of patients with SCA6 was 1.33 ± 1.40 points/year. Additionally, our study demonstrated both the limitations and the advantages of Japan's IDR registry. Finally, the results of this study may have important implications for the planning of future clinical trials that investigate new treatments for the SCAs.

Abbreviations

ADCA: Autosomal dominant cerebellar ataxia; ADL: Activities of daily living; BI: Barthel index; *CACNA1A*: Alpha 1A P/Q type voltage-dependent calcium channel gene; CAG: Cytosine-adenine-guanine; ICARS: International Cooperative Ataxia Rating Scale; IDR: Intractable diseases research; SARA: Scale for the Assessment and Rating of Ataxia; SCA: Spinocerebellar ataxia.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KY: research project execution, statistical analysis design, statistical analysis of data, drafting the manuscript for intellectual content; IY: research project execution, manuscript review and critique; KY: research project execution, manuscript review and critique; KK: research project execution, statistical analysis and critique, manuscript review and critique; KA: research project execution, manuscript review and critique; MI: research project execution, manuscript review and critique; OO: research project execution, manuscript review and critique; SK: research project execution, manuscript review and critique; EI: research project execution, manuscript review and critique; SS: research project execution; YA: research project execution; HS: research project organization, research project execution, manuscript review and critique, study supervision; SK: research project execution, manuscript review and critique; TH: research project execution, manuscript review and critique; GS: research project organization, research project execution, manuscript review and critique; HM: research project organization, manuscript review and critique; ST: research project organization, manuscript review and critique; MN: research project organization, research project execution, manuscript review and critique, study supervision; KN: research project organization, research project execution, statistical analysis design, statistical analysis and critique, manuscript review and critique, study supervision. All authors read and approved the final manuscript.

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INTERNAL MEDICINE

□ CASE REPORT □

An Autopsy Case Involving a 12-year History of Amyotrophic Lateral Sclerosis with CIDP-like Polyneuropathy

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Abstract

Demyelinating polyneuropathy associated with amyotrophic lateral sclerosis (ALS) is quite rare. We herein present the case of a woman patient with a 12-year history of chronic inflammatory demyelinating polyneuropathy (CIDP)-like polyneuropathy who later developed bulbar palsy and respiratory failure. The autopsy findings revealed neuronal loss in the anterior horn and primary motor cortex with degeneration of the corticospinal tracts. Diffuse phosphorylated TAR DNA-binding protein of 43 kDa inclusions were observed in the anterior horn and cerebral cortices, including the temporal lobe. The final diagnosis was ALS with CIDP-like polyneuropathy. Compared with other reports of ALS with CIDP-like polyneuropathy, the present patient was younger and followed a relatively long clinical course, with no upper motor neuron signs.

Key words: amyotrophic lateral sclerosis, chronic inflammatory demyelinating polyneuropathy, autopsy, onion bulb formation, TAR DNA-binding protein of 43 kDa

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Introduction

Amyotrophic lateral sclerosis (ALS) is a slowly progressive neurodegenerative disorder impairing both upper and lower motor neurons in the central nervous system, leading to death from respiratory failure, usually within three to five years of symptom onset (1). On the other hand, chronic inflammatory demyelinating polyneuropathy (CIDP) is an acquired immune-mediated demyelinating polyneuropathy that impairs the peripheral nervous system and shows various distributional patterns (2). Recently, some reports of ALS accompanied by CIDP-like polyneuropathy have been published (3-7). Although the pathological characteristics and treatment responses varied among the reported cases, the findings could represent a possible novel phenotype of motor neuron disease.

In this report, we present the case of a Japanese woman with autopsy-proven ALS associated with CIDP-like polyneuropathy whose disease onset was relatively young and disease progression was relatively slow compared to that observed in previously reported patients. This is an important case for understanding the range of the disease spectrum of this rare condition.

Case Report

The patient had no remarkable family or past medical history except for iron-deficiency anemia. At 37 years of age, she noticed a tendency to stumble due to slight weakness in the feet and cramps in the calves. At 39 years of age, she developed difficulty in performing dorsiflexion of the left

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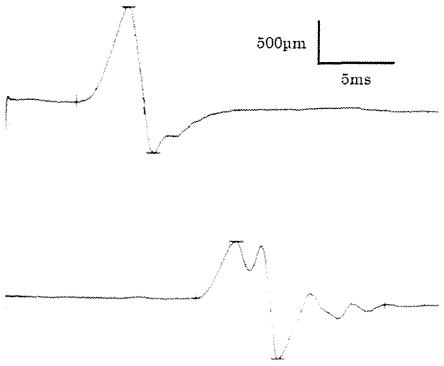

Figure 1. **Nerve conduction studies in left tibial nerve. Temporal dispersion with normal distal latency was present. The amplitudes were smaller than the lower limit of normal values, but sufficient for fulfilling the electro-diagnostic criteria of CIDP.**

foot with hypoesthesia and a tingling sensation in the bilateral hands. The muscle weakness in her legs gradually worsened, and she was referred to our hospital at 42 years of age. A neurological examination revealed distal dominant muscle weakness in the bilateral legs with slight atrophy, particularly in the left leg. Hyperalgesia with a multifocal distribution was found in the bilateral fingertips and areas innervated by peroneal nerves, sural nerves and the right plantar nerve. The left Achilles tendon reflex was absent. A blood test showed slight elevation of creatine kinase (258 IU/L). The cerebrospinal fluid was normal, with a normal protein level of 0.25 g/L. Anti-ganglioside antibodies, including anti-GM1 antibodies were negative. Brain MRI and spine MRI with gadolinium enhancement were normal, with no swelling or T2-hyperintense foci in the nerve roots.

A nerve conduction study (NCS) performed at 43 years of age revealed loss of the F-wave in the left median, tibial and peroneal nerves. In addition, temporal dispersion was found in the left tibial nerve (Fig. 1). An epon-embedded specimen of the left sural nerve (Fig. 2a) revealed normal-density myelinated fibers (7,210/mm^2) with scattered thinly myelinated fibers and a small number of areas of onion bulb formation (Fig. 2b). The unmyelinated fibers were mostly spared, without apparent axonal degeneration (Fig. 2c). The teased-fiber method revealed approximately 10% segmental demyelination and remyelination. Neither duplication nor deletion in the peripheral myelin protein-22 (PMP22) gene were found on a fluorescence in situ hybridization (FISH) analysis.

Since the patient was suspected to have demyelinating polyneuropathy, intravenous immunoglobulin (IVIG) therapy (0.4 g/kg daily for five days) was administered, which achieved a partial effect on the left foot drop. The muscle strength of the left tibialis anterior increased from 2 to 3 according to a manual muscle test (MMT), and the patient's gait improved. She also reported alleviation of the sensory disturbance in both hands. At that time, the European Federation of Neurological Societies/Peripheral Nerve Society (EFNS/PNS) criteria for CIDP were met based on one electrodiagnostic criterion with two supportive criteria (8). She was discharged from the hospital on prednisolone therapy (20 mg orally per day).

Over the ensuing 12 months, the patient's muscle strength gradually deteriorated. The bilateral patellar tendon reflexes were temporarily increased; however, no pathological reflexes were observed. A second cycle of IVIG therapy was administered at 43 years of age, which was again partially effective and improved the left leg strength from 2 to 3 on MMT, with the improvement lasting for the next several months. At 44 years of age, left arm weakness appeared, followed by right arm weakness. A neurological examination revealed muscle weakness in all four limbs with glove-and-stocking type hypesthesia. The IVIG therapy partially improved the muscle strength of the upper limbs, and the sensory disturbance was also slightly ameliorated, IVIG therapies was subsequently consecutively administered two to three times a year; however the efficacy gradually diminished. We were unable to confirm an objective neurological improvement after 45 years of age. Methylprednisolone pulse therapy and cyclosporin-A were also administered, although neither were effective. By 45 years of age, the weakness and muscle atrophy in all extremities had further progressed in association with the absence of deep tendon reflexes, and the patient was no longer able to stand. At 46 years of age, tongue atrophy with fasciculation, dysarthria and dysphagia emerged. The bulbar symptoms further worsened, and percutaneous endoscopic gastrostomy was performed. At 47 years of age, the muscle strength in all extremities was 1-2 on MMT, and the respiratory disturbance gradually progressed. The patient refused treatment with a ventilator and died of respiratory failure at 48 years of age, nearly 12 years after the onset. No cognitive deficits were noted throughout the patient's clinical course, and no difficulties in executive decision making were observed.

Autopsy findings

The brain was of normal weight (1,220 g) with no marked atrophy or deformities. The structures of the cerebellum and brain stem also appeared to be within the normal limits. Routine Hematoxylin and Eosin (H&E) staining and Kluver-Barrera staining revealed preserved neurons in the hippocampus and cerebral cortices. On immunostaining, cytoplasmic inclusions of phosphorylated TAR DNA-binding protein of 43 kDa (TDP-43) were observed in the right frontal lobe cortex, right subiculum, granule cells of the hippocampus and adjacent temporal lobe cortex (Fig. 3a). In the motor cortices, loss of Betz cells and gliosis were observed on H&E staining. In the brain stem, partial loss and atrophy of motor neurons in the hypoglossal nuclei were found. The remaining neurons were negative for phosphorylated TDP-43.

The spinal cord was almost normally shaped, with no apparent systematic atrophy (Fig. 3b); however, stainability was mildly reduced in the lateral funiculi. The spinal ventral

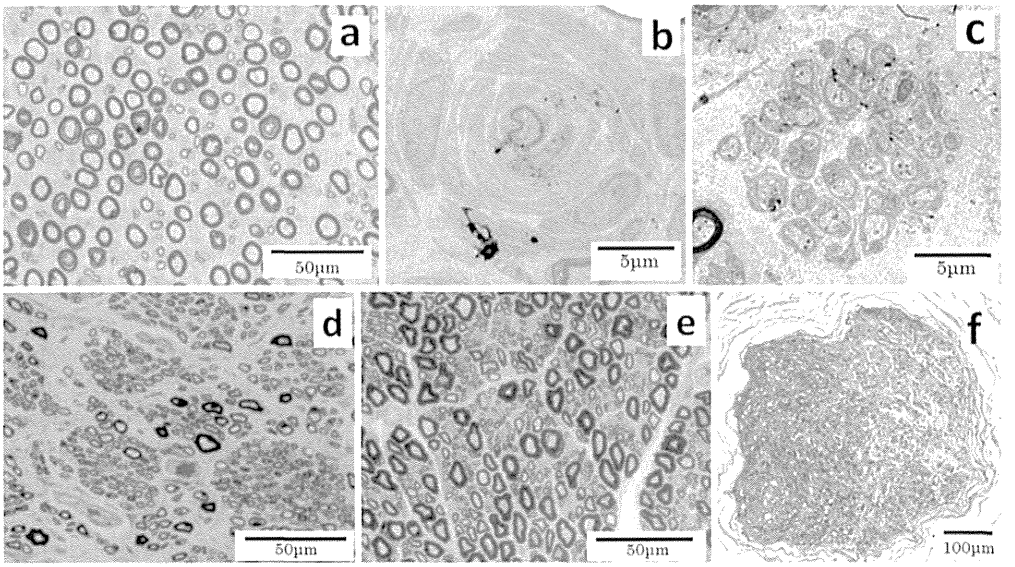

Figure 2. **Pathological findings in peripheral nervous system. (a) Left sural nerve biopsy. There were scattered thinly myelinated fibers. Toluidine blue staining. (b) Electron micrography of onion bulb formation showed four to five concentric layers of Schwann cell processes. A few onion bulb formations, like this one, were observed in each nerve fascicle. (c) Electron micrography of unmyelinated fibers. Fibers were spared without axonal degeneration. (d) Spinal ventral roots. Large myelinated fibers were markedly reduced in number. Some remaining large fibers were thinly myelinated. Toluidine blue staining. (e) Spinal dorsal roots. Fibers were mostly spared compared to the ventral roots. (f) Lumbar plexus. Focal axonal loss, probably reflecting degeneration of motor neurons, was found. Elastica-Masson staining.**

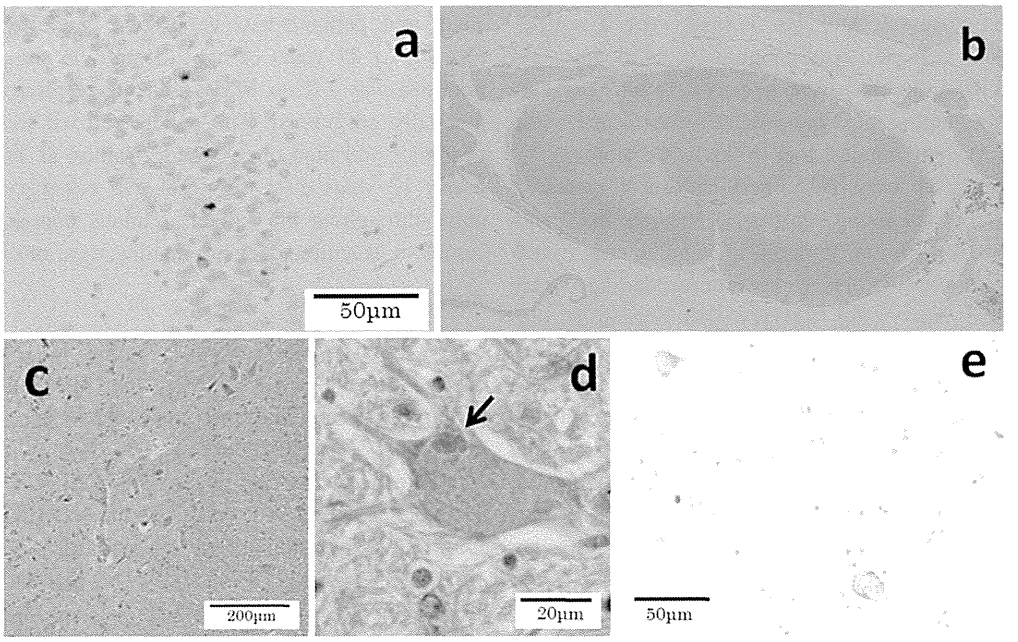

Figure 3. **Pathological findings in central nervous system. (a) Dentate gyrus of the hippocampus. Phosphorylated TDP-43-positive inclusions were observed in the cytoplasm of granule cells. (b) Cervical cord at C6 level. Myelin pallor of the lateral corticospinal tracts was observed by Kluver-Barrera staining. (c) Anterior horn of the lumbar cord. Severe neuronal loss was observed. Hematoxylin and Eosin (H&E) staining. (d) Anterior horn of the lumbar cord. A Bunina body was observed in the cytoplasm (arrow). H&E staining. (e) Anterior horn of the lumbar cord. Granular phosphorylated TDP-43 immunoreactivities were diffusely observed in the cytoplasm of the remaining lower motor neurons.**

Table. **Clinical Characteristics, Pathological Findings, and Effectiveness of IVIG Therapy in 13 Previously Reported Cases of ALS with CIDP-like Polyneuropathy and the Present Case**

Ref	Onset age, sex	Duration [months]	Fas.	Sens. Dist.	Peripheral nerve pathology	CNS pathology	TDP-43 or Bunina body (C/S)	IVIG (E/I)
3	44, M (FALS)	20	+	-	MCI	U, L	S	I
3	63, M (FALS)	13	+	-	n.d.	n.d.	n.d.	I
3	52, M	38	+	-	n.d.	n.d.	n.d.	I
4	65, F	45	-	-	n.d.	n.d.	n.d.	I
4	65, M	36	-	-	Dmy, AD	n.d.	n.d.	I
4	57, F	77	-	+	n.d.	n.d.	n.d.	I
4	68, F	38	+	+	Dmy	n.d.	n.d.	E
5	66, M	59	-	+	OB, Dmy	n.d.	n.d.	I
5	53, F	53	+	-	n.d.	n.d.	n.d.	E
5	50, M	50	+	-	OB, Dmy	n.d.	n.d.	I
6	70, M (CMT1A)	43	-	+	OB, Dmy, MCI	U, L	S	E
7	45, M	48	-	+	OB, Dmy	n.d.	n.d.	E
7	68, M	11	+	-	Dmy	U, L	S	E
(the present case)	37, F	142	-	+	OB, Dmy	U, L	C, S	E

Ref: reference number, FALS: familial amyotrophic lateral sclerosis, CMT1A: Charcot-Marie-Tooth disease type 1A, M: male, F: female, Fas: fasciculation, Sens. Dist.: sensory disturbance, MCI: endoneurial mononuclear cell infiltration, OB: onion bulb formation, Dmy: segmental demyelination, AD: axonal degeneration, CNS: central nervous system, U: (abnormalities in) upper motor neuron, L: (abnormalities in) lower motor neuron, TDP-43: TAR DNA-binding protein of 43 kDa, C/S: (TDP-43 or Bunina body in) cerebrum and/or spine, IVIG: intravenous immunoglobulin therapy, E/I: effective or ineffective, n.d.: not done.

roots were somewhat atrophic compared to the dorsal roots. Although the anterior horn was not atrophied at any level of the spine, anterior horn cells were diffusely degenerated and reduced in number at the cervical (C6), thoracic (T4) and lumbar (L3) levels of the cord (Fig. 3c). A Bunina body was detected in a remaining motor neuron at the L3 level (Fig. 3d). Phosphorylated TDP-43-positive cytoplasmic inclusions were found in the anterior horn (Fig. 3e) on immunohistochemical staining. Although the volume of the lateral corticospinal tract was mostly preserved, myelin pallor was observed on Kluver-Barrera staining.

Peripheral nerves were sampled from the lumbar ventral and dorsal roots, lumbar plexus and proximal sciatic nerves. In the ventral roots, large myelinated fibers were reduced in number, and many small myelinated fibers were found (Fig. 2d). In the dorsal roots, the nerve fibers were mostly spared, showing a small number of thinly myelinated fibers (Fig. 2e). In the lumbar plexus, focal axonal loss was observed in some fascicles (Fig. 2f). There were some thinly myelinated fibers in the proximal sciatic nerves. No apparent infiltration of inflammatory cells was noted in the examined peripheral nerves. Muscles were sampled from the tongue, diaphragm and iliopsoas, all of which demonstrated neurogenic atrophy with fat infiltration.

Discussion

Because the patient was diagnosed with CIDP based on the nerve biopsy and NCS findings, the advent of bulbar palsy and respiratory failure, which are rare manifestations of CIDP (9), in the last two years was unexpected.

To our knowledge, the coexistence of ALS and demyelinating polyneuropathy resembling CIDP has been reported in 13 patients (3-7). Their clinical and pathological findings are summarized with those of the present patient in Table. The median age of onset was 64 years (range: 37-70 years old), and the median duration of disease (onset to respiratory failure) was 44 months (range: 11-142 months). ALS was diagnosed in all cases according to the El Escorial criteria (10) and/or autopsy findings (four cases). On the other hand, CIDP-like polyneuropathy was diagnosed on the initial admission using established diagnostic criteria (8, 11) based on NCS findings in most cases. Sural nerve pathology was described in eight cases, and demyelination was confirmed in all cases. Mononuclear cell infiltration in the peripheral nerves was observed in two cases, one at autopsy (5) and the other on a biopsy and at autopsy; the latter case involved Charcot-Marie-Tooth disease (6). All 14 patients received IVIG therapy, and some neurological improvements were reported in six cases. The effects of treatment lasted from a few weeks to several months, and addi-

tional courses of IVIG therapy were ineffective, except in the present case. Some patients appeared to develop symptoms of ALS after the period of clinical manifestations of CIDP-like polyneuropathy, while others showed symptoms suggestive of ALS, such as upper motor neuron signs, on the initial presentation. Among the latter patients, the manifestations on the initial presentation were consistent with those of ALS, except for the NCS findings in some cases, including two patients with familial ALS (3). Whether CIDP-like polyneuropathy evolved as an epiphenomenon of ALS or existed as a comorbidity of ALS was unclear in most of the cases.

Compared with the 13 previously reported patients, our patient was younger at disease onset, and the duration of disease was especially long. The lack of upper motor neuron signs made the diagnosis more difficult. Although a small number of ALS patients are known to exhibit slow disease progression of up to 10 years or more (12-14) and present with only lower motor neuron signs clinically (14, 15), the neuropathy may have partially contributed to the present patient's symptoms, which improved with IVIG therapy. After 45 years of age, when the IVIG therapy no longer achieved any objective improvements, the muscle weakness may have been attributable to ALS, because we did not find any typical CIDP lesions at autopsy, such as onion bulb formation or inflammatory infiltration, although we can not exclude the possibility that such lesions existed in the peripheral nervous system at sites other than those we were able to examine.

Although it is difficult to infer a causal relationship between the CIDP-like polyneuropathy and ALS in the present patient, this and previously reported cases suggest that some patients with ALS exhibit forms of demyelinating polyneuropathy that fulfill the diagnostic criteria for CIDP at the initial presentation. Such types of neuropathy can be partially improved with IVIG therapy, although the final outcome depends on the progression of ALS. Diagnosing ALS clinically is especially difficult if the disease course is prolonged and upper motor neuron signs are absent, as observed in the present case.

The authors state that they have no Conflict of Interest (COI).

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ORIGINAL ARTICLE

Confirmatory double-blind, parallel-group, placebo-controlled study of efficacy and safety of edaravone (MCI-186) in amyotrophic lateral sclerosis patients

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Abstract

Our objective was to confirm the efficacy and safety of edaravone in amyotrophic lateral sclerosis (ALS) patients. We conducted a 36-week confirmatory study, consisting of 12-week pre-observation period followed by 24-week treatment period. Patients received placebo or edaravone i.v. infusion over 60 min for the first 14 days in cycle 1, and for 10 of the first 14 days during cycles 2 to 6. The efficacy primary endpoint was changed in the revised ALS functional rating scale (ALSFRS-R) scores during the 24-week treatment. Patients were treated with placebo ($n = 104$) and edaravone ($n = 102$). Changes in ALSFRS-R during the 24-week treatment were -6.35 ± 0.84 in the placebo group ($n = 99$) and -5.70 ± 0.85 in the edaravone group ($n = 100$), with a difference of 0.65 ± 0.78 ($p = 0.411$). Adverse events amounted to 88.5% (92/104) in the placebo group and 89.2% (91/102) in the edaravone group. In conclusion, the reduction of ALSFRS-R was smaller in the edaravone group than in the placebo group, but efficacy of edaravone for treatment of ALS was not demonstrated. Levels and frequencies of reported adverse events were similar in the two groups.

Key words: *Amyotrophic lateral sclerosis, ALSFRS-R, edaravone, placebo, randomized trial*

Introduction

Amyotrophic lateral sclerosis (ALS) is a refractory and progressive disease that causes selective degeneration of upper and lower motor neurons (1). Median survival from onset to death in ALS is reported to vary from 20 to 48 months (2).

Oxidative stress has been considered to be involved in the onset and progression of ALS (3). An established marker of oxidative stress is 3-nitrotyrosine (3NT), formed by reaction of the free radical peroxynitrite with tyrosine residues. A significant increase of 3NT was reported in spinal cord of transgenic mice expressing mutated SOD1 (4) and in autopsied spinal cord of FALS patients with genetic mutation of SOD1 and sporadic ALS (SALS) patients (5). 3NT was found in motor neurons (6,7) and was elevated in cerebrospinal fluid of SALS patients (8). Moreover, oxidative stress induces nuclear translocation and activation of Nrf-2, a transcription factor that generates an anti-oxidative response (9). Nrf-2 translocation also occurs in mutant TDP-43 transfected cultured motor neuron cell lines (10–12). Consequently, drugs that eliminate free radicals might protect motor neurons from oxidative stress and free radical damage in ALS patients.

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Edaravone (MCI-186, 3-methyl-1-phenyl-2-pyrazolin-5-one, Mitsubishi Tanabe Pharma Corporation, Tokyo, Japan) is a free radical scavenger approved for treatment of acute cerebral infarction in Japan in 2001 (13). Edaravone eliminates lipid peroxides and hydroxyl radicals during cerebral ischemia and protects nerve cells within or around the ischemic region from free radical damage (14–16). Beneficial effects of edaravone have been reported in wobbler mice with ALS-like symptoms (17) and in ALS-model animals (18,19).

A phase II trial was conducted to investigate the safety and efficacy of edaravone in ALS patients, and found that progression of motor dysfunction was slowed and no clinically significant adverse drug reactions occurred. The level of 3NT was low in cerebrospinal fluid of almost all patients in the phase II trial, suggesting that edaravone could protect neuronal cells from oxidative stress (20). Therefore we designed a clinical trial to confirm the efficacy and safety of edaravone in ALS patients.

Methods

Standard protocol approvals

Twenty-nine sites in Japan participated in the study between May 2006 and September 2008. An institutional review board at each site approved the study protocol. The study was conducted in compliance with Good Clinical Practice (GCP). All participants provided written informed consent before the pre-observation stage. The study sponsor was Mitsubishi Tanabe Pharma Corporation. The study is registered in ClinicalTrials.gov with a registration number NCT00330681.

Patients

Inclusion criteria were: age 20–75 years; diagnosis of 'definite', 'probable' or 'probable laboratory-supported' ALS (21,22) according to the revised Airlie House diagnostic criteria; forced vital capacity (FVC) of at least 70%; duration of disease within three years; and change in revised ALS functional rating scale (ALSFRS-R) (23,24) score during the 12-week pre-observation period of –1 to –4 points. Patients also had a Japanese ALS severity classification (25) of 1 or 2. (The Japanese ALS severity classification score ranges from 1 to 5 according to the severity classification of the Specified Disease Treatment Research Program for ALS of the Ministry of Health, Labor and Welfare of Japan. Severity Classification: 1) able to work or perform housework; 2) independent living but unable to work; 3) requiring assistance for eating, excretion or ambulation; 4) presence of respiratory insufficiency, difficulty in coughing out sputum or dysphagia; and 5) using a tracheostomy tube, tube feeding or tracheostomy positive pressure ventilation.)

Exclusion criteria were: reduced respiratory function and complaints of dyspnea; complications that may substantially influence evaluation of drug efficacy, such as Parkinson's disease, schizophrenia and dementia; complications that require hospitalization, including liver, cardiac and renal diseases; infections that require antibiotic therapy; deteriorated general condition as judged by investigators; renal dysfunction with creatinine clearance of 50 ml/min or below within 28 days before treatment; and undergoing cancer treatment.

Patient eligibility was assessed with inclusion and exclusion criteria at the start and end of pre-observation.

Administration regimen of riluzole was required not to be changed during the study.

Study medication

Mitsubishi Tanabe Pharma Corporation provided the investigational drugs in ampoules. Only authorized personnel, independent of the sponsor and investigators, had access to the key code until unblinding. The dose of edaravone was 60 mg per day, which was indicated to show efficacy in the phase II trial (20), and placebo was chosen since no suitable comparator drug for ALS has been approved. Saline (placebo) or edaravone was administered once daily by i.v. infusion over 60 min.

Design

After the 12-week pre-observation period, eligible patients were randomized to placebo or edaravone group. Dynamic allocation was used to minimize the effects of the following three factors, which may substantially influence the evaluation of edaravone:

- Factor 1: change in ALSFRS-R score during pre-observation period: two categories: –4, –3 or –2, –1.
- Factor 2: initial symptom: two categories – bulbar or limb.
- Factor 3: use of riluzole: two categories – yes or no.

The study period was 36 weeks, consisting of a 12-week pre-observation period before the start of the first cycle, followed by a 24-week treatment period (Figure 1).

A single treatment cycle consisted of 14 days of study drug administration period followed by a 14-day observation period. Study drugs were administered every day for 14 days in the administration period of the first cycle, and for 10 out of 14 days in the administration periods of cycles 2 to 6. The end of the administration period in each cycle was followed by a 14-day observation period.

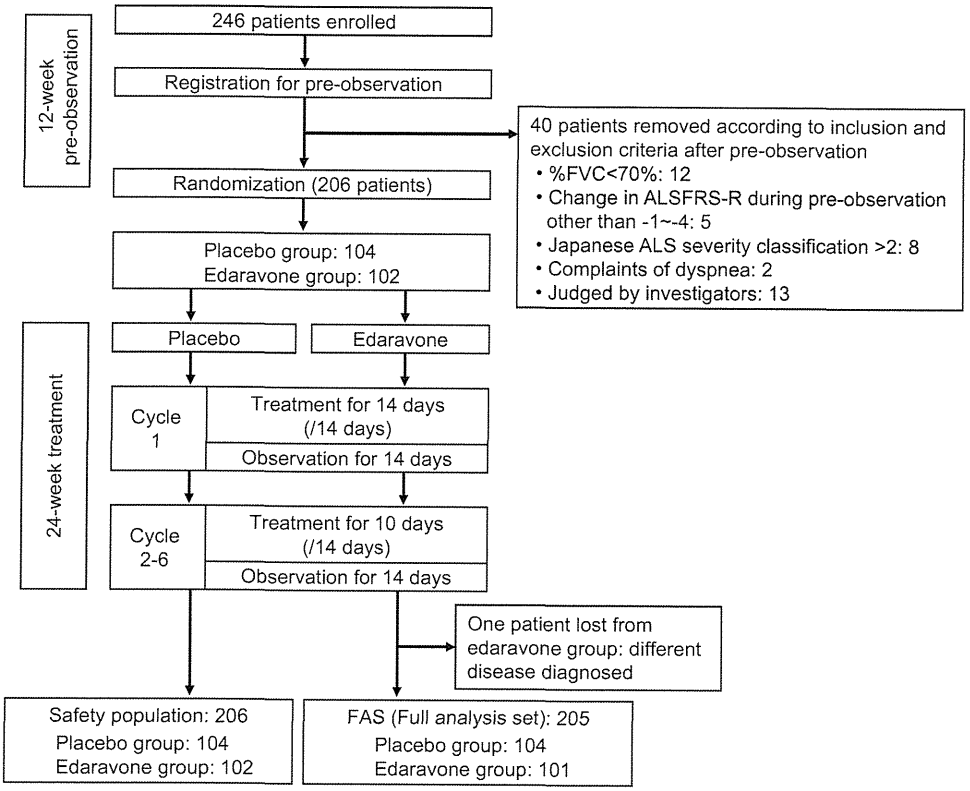

Figure 1. Trial profile.

Efficacy evaluation

Primary efficacy endpoint was the change in ALSFRS-R score. Secondary endpoints were: changes of FVC, grip strength (left/right mean), pinch strength (left/right mean), Modified Norris Scale score (26,27), ALSAQ-40 (ALS Assessment Questionnaire) (28,29), and time to death or a specified state of disease progression (incapable of independent ambulation, loss of function in upper limbs, tracheotomy, artificial respirator with intubation, or tube feeding). The evaluations were carried out at the following times: before pre-observation, before the start of the first treatment cycle and at the end of each treatment cycle (after 14 days observation and before the first dosage of the next cycle).

Safety evaluation

Safety was assessed in terms of number and severity of adverse events (AE), adverse drug reactions and the results of clinical laboratory tests and sensory tests. Serious adverse events were identified from the adverse events according to the GCP guideline.

Statistical analysis

Based upon the experience of the phase II trial (20), we considered that it would be difficult to enroll more than 100 patients per group for the trial and the target number of patients for enrollment was set at 200. In the phase II trial, the difference of the change of ALSFRS-R between the edaravone and placebo groups was 2.2 for patients with matched severity and dose to those of the present study; and on the assumption of a standard deviation of 5.2, the statistical power of this study can be calculated as 85% when 100 patients per group were enrolled.

The primary population used for the efficacy analysis was the full analysis set (FAS). For ALSFRS-R scores, analysis of covariance (ANCOVA) was performed on the change in score during treatment, defined as the difference between the score before the start of the first treatment cycle (before treatment) and the score at two weeks after the end of the sixth treatment cycle (after treatment). Three factors were used for dynamic allocation as covariates, after which the inter-group difference was assessed. Repeated measures analysis of variance was also performed using the treatment group, period, and interaction between treatment group and period (treatment group × period) as design factors, and baseline value and the three factors used for dynamic allocation as covariates, after which the inter-group difference was assessed. Compound symmetry was assumed as a covariance structure of repeated measurement. Edaravone efficacy would be verified if a significant inter-group difference were found in at least one of the above analyses. A two-sided level of significance of 5% and a two-sided 95% confidence interval were chosen for interpretation of main effect. A two-sided level of significance of 15% was chosen for

determining the existence of effect of interaction. A stratified analysis was also performed on the changes of ALSFRS-R score by diagnostic category. The level of significance for differences of patient characteristics was set at 15%.

ANCOVA and repeated measures analysis of variance were similarly performed on the secondary endpoints. Time to death or a specified state of disease progression was defined as an event and the other endpoints were followed until cut-off. A stratified, generalized Wilcoxon test and log-rank test were performed using the change in ALSFRS-R score during the pre-observation period as a stratification factor. For patients with more than one event, the onset date of the first event was defined as the survival time. In censored cases, the cut-off date was the end date of observations.

For patients with missing data at 24 weeks after starting treatment, the last observation carried forward (LOCF) method was applied to impute missing data. Patients who completed the third cycle were eligible for LOCF.

To evaluate safety, AE and adverse drug reactions were assessed in the safety population. Proportions of AE, adverse drug reactions, serious adverse events (SAE) and serious adverse drug reactions were calculated and compared between the groups using Fisher's exact test. A two-sided level of significance of 5% and a two-sided 95% confidence interval were chosen for interpretation. Statistical analysis was performed using SAS software (version 9.1, SAS Institute, Cary, NC).

Results

Subject background

Two hundred and forty-six patients were prospectively registered. After the 12-week pre-observation period, 40 patients were excluded according to the inclusion and exclusion criteria, and the remaining 206 patients were randomized (Figure 1).

The FAS included 205 patients after exclusion of one patient who was diagnosed with a different disease. For the safety evaluation, the number of patients in the safety population was 206, which included all patients treated with the study medication. The treatment was discontinued for 23 patients (edaravone group: patients' request 5, AE 3, tracheotomy 1; placebo group: patients' request 5, AE 6, tracheotomy 2, protocol violation 1). There was no imbalance between the groups in either analysis set on discontinuation (FAS: $p = 0.378$; safety population: $p = 0.377$).

All patients in the safety population received at least 80% of the assigned dosages of study drug.

Patient characteristics are summarized in Table I. Among the patient characteristics, those for which inter-group differences were found at a significance level below 15% were the duration of disease ($p = 0.104$, paired t-test), ALSFRS-R score before pre-observation ($p = 0.065$, paired t-test), and ALSFRS-R score at the start of the first cycle ($p = 0.146$, paired t-test).

Table I. Subject demographic characteristics.

Item	Placebo (104) *n* (%)	Edaravone (101) *n* (%)
Gender		
male	69 (66.3)	63 (62.4)
Initial symptom		
bulbar	20 (19.2)	18 (17.8)
limb	84 (80.8)	83 (82.2)
Diagnosis (El Escorial revisited)		
definite	21 (20.2)	29 (28.7)
probable	54 (51.9)	52 (51.5)
probable laboratory-supported	28 (26.9)	20 (19.8)
possible	1 (1.0)	0 (0.0)
The Japanese severity classification		
grade 1	40 (38.5)	36 (35.6)
grade 2	64 (61.5)	65 (64.4)
Use of riluzole		
yes	92 (88.5)	90 (89.1)
Change in ALSFRS-R score during pre-observation		
−4, −3	32 (30.8)	29 (28.7)
−2, −1	72 (69.2)	72 (71.3)
Item	Placebo (104) median (min-max)	Edaravone (101) median (min-max)
Age (years old)	58.5 (28–75)	58.0 (29–73)
Body weight (kg)	57.0 (37–109)	57.0 (35–77)
Duration of disease (years)	1.20 (0.3–3.0)	1.30 (0.4–2.9)
ALSFRS-R score before pre-observation	44.0 (35–48)	43.0 (31–48)
ALSFRS-R score before treatment period	42.0 (32–47)	41.0 (29–47)

ALSFRS-R: the revised amyotrophic lateral sclerosis functional rating scale.

Table II. Change in endpoints during treatment.

	Change in endpoints during treatment (ANCOVA)				Repeated-measures analysis			
	Adjusted mean change LS Mean ± S.E.		Inter-group difference in adjusted mean change LS Mean ± S.E. (95% C.I.)	p value	Adjusted mean LS Mean ± S.E.		Inter-group difference in adjusted mean LS Mean ± S.E. (95% C.I.)	p value
	Placebo	Edaravone			Placebo	Edaravone		
Primary endpoint								
ALSFRS-R	−6.35 ± 0.84 (99)	−5.70 ± 0.85 (100)	0.65 ± 0.78 (−0.90 – 2.19)	0.411	37.43 ± 0.46	38.08 ± 0.47	0.65 ± 0.44 (−0.22 – 1.52)	0.141
Secondary endpoint								
%FVC	−17.49 ± 2.39 (99)	−14.57 ± 2.41 (100)	2.92 ± 2.24 (−1.49, 7.33)	0.193	87.30 ± 1.56	88.56 ± 1.59	1.26 ± 1.46 (−1.63, 4.15)	0.390
Grip strength	−5.71 ± 0.69 (99)	−4.81 ± 0.69 (100)	0.89 ± 0.64 (−0.37, 2.16)	0.165	13.22 ± 0.42	13.83 ± 0.43	0.60 ± 0.40 (−0.18, 1.38)	0.130
Pinch strength	−1.03 ± 0.15 (99)	−0.83 ± 0.15 (100)	0.20 ± 0.14 −0.08, 0.48)	0.165	2.62 ± 0.11	2.83 ± 0.11	0.21 ± 0.10 (0.01, 0.41)	0.038
Modified Norris scale	−16.15 ± 2.00 (97)	−14.12 ± 2.05 (95)	2.03 ± 1.89 (−1.69, 5.75)	0.284	NA	NA	NA	NA
ALSAQ40	19.13 ± 3.79 (95)	19.60 ± 3.82 (95)	0.48 ± 3.50 (−6.44, 7.39)	0.892	NA	NA	NA	NA

ALSFRS-R: interaction between treatment group and period ($p = 0.915$). ALSFRS-R: the revised amyotrophic lateral sclerosis functional rating scale. NA: not applicable. For Modified Norris scale and ALSAQ40, repeated measures analysis was not conducted

Efficacy

The results of ANCOVA for the change of ALSFRS-R score during treatment and the results of repeated measures analysis of variance are shown in Table II. In both analyses, no significant inter-group difference was observed.

The changes in ALSFRS-R score during treatment according to diagnostic category, i.e. 'definite', 'probable' and 'probable laboratory-supported', are shown in Figure 2.

The results of secondary endpoints are presented in Table II. The pinch strength analyzed by repeated measures analysis of variance showed a statistically significant difference, as there was no interaction between the treatment group and period ($p = 0.292$). The other endpoints did not show a significant difference.

The proportion of events of death or a particular state of disease progression was documented in 27 patients in the placebo group (14 patients with –4, –3 change in ALSFRS-R score during pre-observation, and 13 patients with –2, –1 change) and 32 in the edaravone group (12 patients with –4, –3 change and 20 patients with –2, –1 change). There was no significant inter-group difference (stratified log-rank test: $p = 0.381$, stratified generalized Wilcoxon test: $p = 0.399$).

Safety

The proportion of AE reported in the safety population was 88.5% in the placebo group and 89.2% in the edaravone group. All AE and SAE with a proportion of at least 5% in either group are listed (Table III). The inter-group difference in proportion with 95% confidence interval is 0.8% (–7.8% to 9.4%). There were no significant inter-group differences in the proportion of AE ($p = 1.000$), and in adverse drug reactions ($p = 0.349$). The proportion of SAE was 23.1% in the placebo group and 17.6% in the edaravone group. Two cases of respiratory failure in the placebo group resulted in death; in the edaravone group, there were three deaths (two cases of respira-

Figure 2. Change of ALSFRS-R score during treatment by diagnostic category. ALSFRS-R: the revised amyotrophic lateral sclerosis functional rating scale.