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Appendix B. Supplementary data

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

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ABSTRACT: Spinal and bulbar muscular atrophy (SBMA) is an adult-onset motor neuron disease caused by a CAG repeat expansion in the androgen receptor gene. Because the progression of SBMA is slow, it is plausible to identify biomarkers that monitor disease course for therapeutic development. To verify whether the 6-min walk test (6MWT) is a biomarker of SBMA, we performed the 6MWT in 35 genetically confirmed patients and in 29 age-matched healthy controls. The walk distance covered within 6 min (6MWD) was significantly less in SBMA than it was in controls (323.3 ± 143.9 m and 637.6 ± 94.2 m, respectively; $P < 0.001$). In test–retest analysis, the intraclass correlation coefficient for the 6MWD was high in SBMA patients ($r = 0.982$). In a 1-year follow-up the 6MWD significantly decreased at a rate of 11.3% per year. Our observations suggest that the 6MWT is a biomarker that can be used to monitor progression of motor impairment in SBMA.

Muscle Nerve **38:** 964–971, 2008

WALKING CAPACITY EVALUATED BY THE 6-MINUTE WALK TEST IN SPINAL AND BULBAR MUSCULAR ATROPHY

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Accepted 12 May 2008

Spinal and bulbar muscular atrophy (SBMA) is a hereditary lower motor neuron disease that affects adult males exclusively. It has a prevalence of 1–2 per 100,000 of the total population.[13,20,28] The cause of SBMA is an aberrant elongation of a CAG repeat in the androgen receptor (AR) gene. CAG repeats range from 9 to 36 in normal subjects, but 38 to 62 repeats are found in SBMA patients.[3,22,31,32] CAG repeat expansion has also been detected in Huntington's disease and several forms of spinocerebellar ataxia.[14] The main symptoms of SBMA are weakness and atrophy of the bulbar, facial, and limb muscles. The onset of weakness is usually between 30 and 60 years, followed by slow progression of neuromuscular symptoms.[5] The onset of symptoms and clinical features of SBMA are dependent on the CAG repeat size, as has been observed in other polyglutamine diseases.[10,31]

Although there is no effective treatment for SBMA, several therapeutic candidates have recently emerged from studies in animal models, and clinical trials have been proposed.[18,19,24,33,36] It is, however, difficult to assess the effects of intervention on true disease endpoints such as the occurrence of pneumonia or the length of time a patient lives free from the use of a wheelchair, because the progression of symptoms is notably slow in SBMA.[5] Therefore, appropriate surrogate endpoints are needed to facilitate the clinical application of animal study results. In this regard, it is important to identify biomarkers of SBMA that reflect the pathogenic processes and can be used as surrogate endpoints. Although nuclear accumulation of mutant AR protein in the scrotal skin has been shown to be a candidate for a histopathological biomarker,[1,6] clinical parameters to evaluate motor function have not been established for SBMA.

The 6 min walk test (6MWT) is one of the most popular clinical tests used for assessment of functional capacity. This test evaluates the global and integrated responses of all the systems involved in walking, including the pulmonary and cardiovascular systems, neuromuscular units, muscle metabo-

Abbreviations: 6MWD, 6-min walk distance; 6MWT, 6-min walk test; ALS, amyotrophic lateral sclerosis; ALSFRS-R, ALS functional rating scale-revised; AR, androgen receptor; PCR, polymerase chain reaction; SBMA, spinal and bulbar muscular atrophy

Key words: spinal and bulbar muscular atrophy; 6-minute walk test; biomarker; exercise test; 6-minute walk duration

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Published online 21 July 2008 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/mus.21077

lism, systemic circulation, peripheral circulation, and blood condition. Because the 6MWT accurately reflects patients' activities of daily living, it has been applied widely for evaluation of functional exercise capacity in cardiopulmonary disorders.[29] It has also been employed to assess functional exercise capacity in neuromuscular diseases such as stroke, Parkinson's disease, cerebral palsy, chronic poliomyelitis, multiple sclerosis, myotonic dystrophy, fibromyalgia, and spinal cord injury.[2,7,8,11,12,16,21,23,25,27] In diseases that affect multiple regions of the nervous system, the 6 min walk distance (6MWD) correlates well with other measurements that evaluate systemic motor function such as muscle strength, clinical scores, and health status questionnaires.[16,23,25]

The aim of this study was to evaluate functional exercise capacity in patients with SBMA using the 6MWT. We also investigated the natural history of the 6MWD in order to determine whether it is an appropriate biomarker that can be used as a surrogate endpoint in forthcoming clinical trials.

MATERIALS AND METHODS

Participants. A total of 35 patients with a diagnosis of SBMA confirmed by genetic analysis were included. Patients were included if they were capable of walking independently along a flat corridor with or without the use of a cane or similar equipment. Exclusion criteria included unstable angina or myocardial infarction during the previous month, tachycardia (>120/min), and uncontrolled hypertension (>180/100 mmHg). We also evaluated 29 age-matched control subjects in this study. The first test was performed between May 2006 and June 2007; reevaluation of 24 of the 35 SBMA patients was conducted ≈1 year after the initial test. The remaining cases were excluded from the follow-up evaluation, because they participated in another interventional study after their first 6MWT evaluation. The age-matched controls did not undergo serial testing.

In addition to the 6MWT, we also evaluated general motor function using clinical scales for amyotrophic lateral sclerosis (ALS), such as the Limb Norris Score, the Norris Bulbar Score, the ALS functional rating scale-revised (ALSFRS-R), and grip power. We defined the onset of disease as the time when muscle weakness began, but not when tremor of the fingers appeared. 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. The Institutional Review Board of Nagoya University Graduate School of Medicine approved the study, and all SBMA patients and normal subjects gave their informed consent for the investigation.

Six-min Walk Test. The 6MWT was performed according to the guidelines provided by the American Thoracic Society.[4] Briefly, examiners instructed participants to walk at their own pace as far as possible in 6 min. The patients were allowed to rest when needed. No encouragement was made throughout the test. The total distance walked during 6 min (6-min walk distance: 6MWD) was recorded. Patients with severe weakness were permitted to use a cane or equivalent assistive device if needed. The 6MWT was performed along a long, flat, straight, enclosed corridor with turnaround points at an interval of 30 m. Although the time of day was not the same, all tests were performed indoors with the same lighting and temperature. All patients were instructed to wear sneakers or equivalent shoes. The results were based on a single 6MWT. We did not perform repeated tests or practice sessions, because patients with SBMA have severe fatigability, which might produce unstable data and cause safety problems.[26,30,35] In addition to the 6MWD, we also recorded the Borg scale before and after the 6MWT.

To assess reliability the 6MWT was repeated within 60 days after the first test in 15 patients without informing them of the results of the previous test. Likewise, patients were not allowed to know the distance covered in the first test when they took the 1-year follow-up study.

Genetic Analysis. Genomic DNA was extracted from peripheral blood of SBMA patients using conventional techniques.[32] Polymerase chain reaction (PCR) amplification of the CAG repeat in the AR gene was performed using a fluorescein-labeled forward primer (5′-TCCAGAATCTGTTCCAGAGCGTGC-3′) and a nonlabeled reverse primer (5′-TGGCCTCGCTCAGGATGTCTTTAAG-3′). Detailed PCR conditions and measurement of CAG-repeat size were described previously.[10,32]

Data Analysis. All data are presented as means ± SD. Changes in the 6MWD were compared using a paired *t*-test. Correlations among the parameters were analyzed using Pearson's correlation coefficient. *P*-values less than 0.05 and correlation coefficients (r) greater than 0.4 were considered to indicate significance. Calculations were performed using the statistical software package SPSS 14.0J (SPSS Japan, Tokyo, Japan).

RESULTS

Clinical and Genetic Backgrounds of SBMA Patients. The clinical characteristics of the study population are presented in Table 1A. There were a total of 35 subjects in the study. All participants were male and of Japanese nationality. The duration from onset was assessed at the first notice of motor impairment[5] and this ranged from 1 to 32 years. There was no significant difference between the median CAG repeat length in the present study and those reported previously in SBMA patients.[3,22,32] All patients were ambulatory with or without aid, and none were bedridden or wheelchair-bound. Other complications that required medication were found in 33 (94.3%) out of 35 patients: diabetes mellitus in 12 (34.3%), hyperlipidemia in 23 (65.7%), hypertension in 20 (57.1%), and depression in 3 (8.6%). Ischemic heart disease or pulmonary disorders were not documented in any patients included in this study.

Reliability of the 6MWT in SBMA Patients. To estimate test–retest reliability we performed the 6MWT on 15 randomly chosen SBMA patients on two different occasions at an interval of 29.4 ± 10.9 days. There was no statistical difference between the clinical scores of the patients who underwent test–retest analysis and those of the remaining subjects (Table 1B). In each patient the two tests were conducted by different examiners. As shown in Figure 1, when the two sets of the 6MWT were compared with one another the intraclass correlation coefficient was 0.982 ($P < 0.001$), indicating an excellent test–retest reliability for SBMA patients.

6MWD and Relevant Clinical Parameters in SBMA. To verify that the 6MWT detects motor impairment in SBMA patients we compared the data from a total of 35 cases and 29 age-matched control subjects (Table 2). There was a significant difference ($P < 0.001$) in

Table 1. Clinical and genetic features of SBMA patients.

A Clinical and genetic features	Mean ± SD (range)	*n*
Age at examination (years)	55.8 ± 11.2 (33–74)	35
CAG repeat length in AR gene (number)	48.3 ± 3.5 (42–57)	29*
Duration from onset (years)	9.7 ± 7.1 (1–32)	35
Limb Norris Score (normal score = 63)	52.5 ± 7.3 (34–62)	35
Norris Bulbar Score (normal score = 39)	33.3 ± 4.2 (20–39)	35
ALSFRS-R (normal score = 48)	41.2 ± 3.7 (33–47)	35
Grip power (kg)†	18.7 ± 5.3 (8.3–33.9)	33

B	Followed-up group		Non followed-up group		
Clinical and genetic features	Mean ± SD (range)	*n*	Mean ± SD (range)	*n*	*P*
Age at examination (years)	55.3 ± 10.9 (33–70)	24	56.7 ± 12.3 (34–74)	11	NS
CAG repeat length in AR gene (number)	48.6 ± 3.6 (42–57)	19*	47.7 ± 3.4 (42–52)	10*	NS
Duration from onset (years)	10.3 ± 7.7 (1–32)	24	9.4 ± 5.9 (2–21)	11	NS
Limb Norris Score (normal score = 63)	52.3 ± 7.3 (34–62)	24	52.9 ± 7.7 (39–62)	11	NS
Norris Bulbar Score (normal score = 39)	33.3 ± 4.3 (20–39)	24	33.5 ± 4.1 (25–38)	11	NS
ALSFRS-R (normal score = 48)	41.0 ± 3.5 (35–46)	24	41.5 ± 4.2 (33–47)	11	NS
Grip power (kg) †	19.6 ± 4.3 (12.0–27.2)	22	17.0 ± 6.7 (8.3–33.9)	11	NS

C	Retested group		Non retested group		
Clinical and genetic features	Mean ± SD (range)	*n*	Mean ± SD (range)	*n*	*P*
Age at examination (years)	57.0 ± 11.7 (34–74)	15	54.9 ± 11.0 (33–68)	20	NS
CAG repeat length in AR gene (number)	48.4 ± 3.9 (42–57)	14*	48.3 ± 3.2 (42–53)	15*	NS
Duration from onset (years)	9.3 ± 5.6 (2–21)	15	10.5 ± 8.2 (1–32)	20	NS
Limb Norris Score (normal score = 63)	52.7 ± 7.4 (39–62)	15	52.3 ± 7.5 (34–62)	20	NS
Norris Bulbar Score (normal score = 39)	32.7 ± 5.1 (20–38)	15	33.8 ± 3.4 (26–39)	20	NS
ALSFRS-R (normal score = 48)	41.4 ± 4.1 (33–47)	15	41.0 ± 3.4 (35–46)	20	NS
Grip power (kg) †	18.4 ± 6.3 (8.3–33.9)	15	19.0 ± 4.4 (12.0–27.2)	18	NS

Data are shown as mean ± SD.

**The abnormal elongation of the CAG repeat was confirmed by gene analysis using agarose gel electrophoresis without determining the repeat number in the remaining 6 patients.*

†The average of both hands. The normal control data of male Japanese at 50–54 years: 43.7 ± 6.4 kg. (The report of physical strength and athletic capability surveillance in 2005.)

AR, androgen receptor; ALSFRS-R, ALS functional rating scale-revised; NS, not significant.

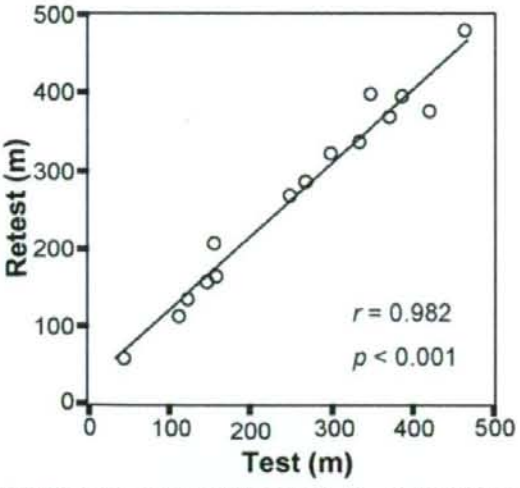

FIGURE 1. Test–retest analysis of the distance walked within 6 min (6MWD). Two sets of 6-min walk tests were carried out within 60 days in 15 SBMA patients who did not know the result of their previous test.

the 6MWD between SBMA patients and the controls. There was no significant difference in the posttest Borg scale, a semiquantitative measure of perceived exercise-related fatigue, between SBMA patients and controls.

We also evaluated motor function of SBMA patients using functional scales and grip power. Because there are no specific motor scores for SBMA we adopted the functional scales used for ALS. The values of the 6MWD correlated well with those of the Limb Norris Score, the Norris Bulbar Score, and the ALSFRS-R (Fig. 2A–C). The value of the 6MWD was inversely correlated with disease duration, although there was no correlation between the 6MWD and grip power (Fig. 2D,E).

Natural History of the 6MWD in SBMA. To delineate the progression rate of walking disturbance the 6MWT was reperformed in a group of 24 SBMA patients at 54.3 ± 6.8 weeks after the initial evaluation. Although follow-up data for the remaining cases was not available because of participation in another interventional study, there was no statistical difference between the backgrounds of the followed subjects and the remaining cases (Table 1C). The 6MWD was significantly decreased in comparison with the distance in the first test ($P = 0.001$), although no motor functional scales showed significant deterioration during the same time period (Table 3). It should be noted that the rate of decline was 11.3 ± 17.6%, which was relatively constant regardless of the walking capacity at the initial evaluation (Fig. 3A). When the patients were stratified by their baseline severity, the change in 6MWD during the follow-up period was larger in the less affected subgroup (Fig. 3B,C).

Based on the natural history of the 6MWD in SBMA, we calculated the sample size for a clinical trial targeting 6MWD, the Limb Norris Score, the Norris Bulbar Score, and ALSFRS-R (Table 4). The sample sizes for a clinical trial using the 6MWT are smaller than those using motor scales, suggesting that it is a more feasible outcome measure than the other clinical scores.

Safety of the 6MWT in SBMA Patients. Throughout the tests, no adverse events such as angina and dyspnea were reported. Although 10 cases walked using a cane and 1 patient walked leaning against a wall, no patients fell or tripped during the tests.

DISCUSSION

This study demonstrates that the 6MWT is a practical, reliable, and safe procedure to measure the walking capacity of SBMA patients. Gait disturbance is the initial symptom in the majority of SBMA patients, and it precedes other health problems such as respiratory failure and dysphagia by ≈10–20 years.[5] Therefore, walking capacity is one of the strongest determining factors of activities of daily living during disease progression in SBMA, implying that the 6MWT appears to be a valuable target for future therapeutic interventions. Although the duration of illness of the patients we tested ranged from 1 to 32 years, our observations suggest that the 6MWT is applicable for patients with various disease durations as long as they are capable of walking.

As a quantitative measure of walking capacity the 6MWT was originally developed for cardiorespiratory and cardiovascular populations. Since then, this test has been applied to various medical conditions including neuromuscular disorders. For example, the 6MWD is strongly correlated with functional

Table 2. Six-min walk distance (6MWD) in SBMA and healthy controls.

	SBMA ($n = 35$)	Healthy controls ($n = 29$)	P
6MWD (m)	323.3 ± 143.9	637.6 ± 94.2	< 0.001
Age at examination (years)	55.8 ± 11.2	52.8 ± 10.7	NS
Borg scale	3.9 ± 2.4	3.2 ± 2.1	NS

Data are shown as mean ± SD.

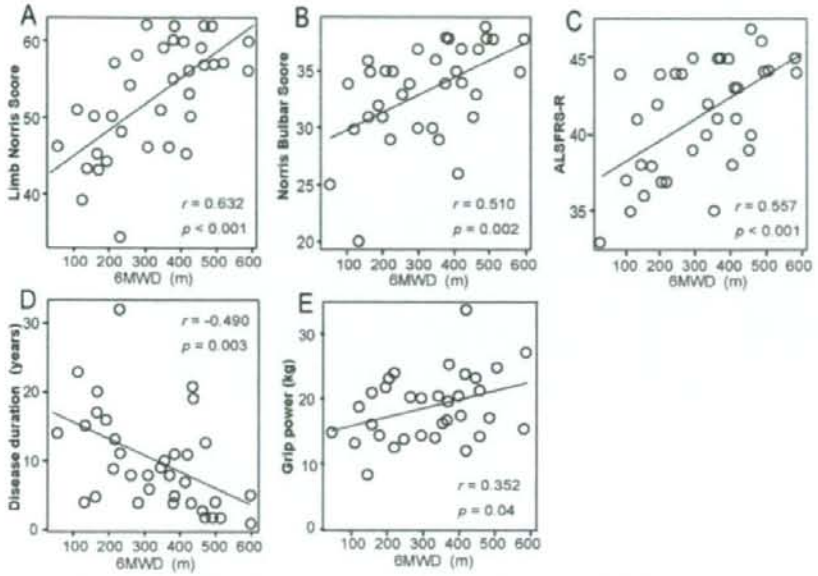

FIGURE 2. Correlation between the 6MWD and other measurements of motor function. **A–C:** The correlation between the 6MWD and general motor function. There was a significant correlation between the 6MWD and motor functional scales such as the Limb Norris Score **(A)**, the Norris Bulbar Score **(B)**, and the ALS functional rating scale-revised (ALSFRS-R, **C**). **D:** The value of the 6MWD was inversely correlated with disease duration. **E:** There was no correlation between the 6MWD and grip power. The value of grip power is shown as the average of left and right hands. 6MWD, six-min walk distance.

scores for balance, strength, and spasticity in post-stroke patients.[12] In patients with multiple sclerosis, the 6MWD is shortened when compared to age-matched healthy controls, reflecting physical function disability.[27] Moreover, the 6MWT has been used as an outcome measurement in various clinical trials for patients with mucopolysaccharidosis, postpolio syndrome, and stroke.[9,15,17,34] Partially due to the small number of patients, there are no established clinical parameters to quantify motor function in SBMA. The total distance covered in 6 min correlated well with motor functional scores in SBMA patients in the present study. Because SBMA is a single gene disorder, various tissues are affected to a similar extent in this disease.[6] This appears to be the reason why 6MWD correlates excellently with clinical scores that reflect disability of other parts of the nervous system affected in SBMA. In addition to muscle weakness, SBMA patients often perceive fatigue during continuous exercise, suggesting that objective measurement to detect the degree of functional endurance is feasible for this disease. Our findings indicate that the 6MWT is a practical examination for measuring functional exercise capacity of patients with SBMA and those with other neurodegenerative diseases.

Table 3. Chronological change in motor function in SBMA.

	n	Initial test	Follow-up	*P*
6MWD (m)	24	351.0 ± 142.8	308.5 ± 132.0	0.001
ALSFRS-R	24	41.0 ± 3.5	39.8 ± 4.0	NS
Limb Norris Score	24	52.3 ± 7.3	50.9 ± 8.2	NS
Norris Bulbar Score	24	33.3 ± 4.3	32.9 ± 4.4	NS

Data are shown as mean ± SD. ALSFRS-R, ALS functional rating scale-revised.

Repeated measurement procedures might result in misleading results, because of practice effect. Therefore, it is important to investigate the reliability of this test in order to determine its feasibility for clinical measurement. Although the 6MWD has been shown to increase only slightly in a second performance a day later, the effect of practice wears off after a week.[4,21] The present study also demonstrated that the reliability of the 6MWT is excellent for SBMA patients, if they are tested within an interval of ≈1 month.

Given the rapid advance of therapeutic developments in animal studies, it is a high priority to search for biomarkers to determine disease severity and

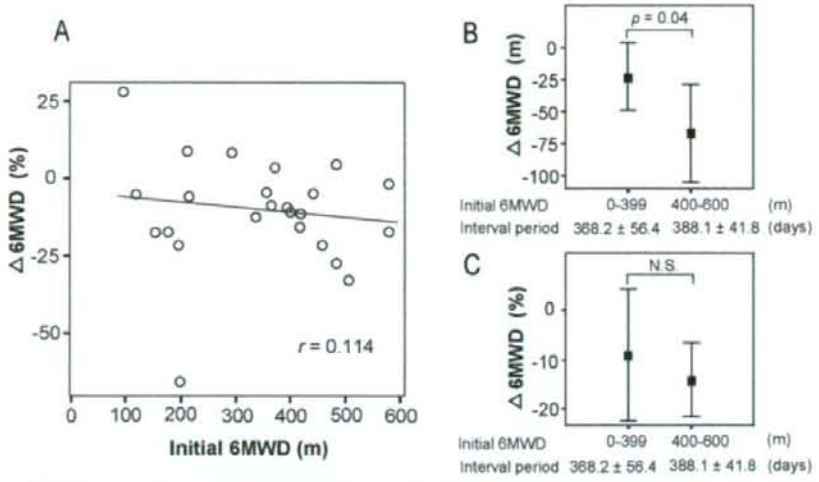

FIGURE 3. The relationship between the change in the 6MWD and the distance in the initial test. **A:** The decline in the 6MWD did not correlate with the result of the initial evaluation. **B,C:** The decline in the 6MWD for each of the severity groups (**B,** actual change; **C,** % change). There was no statistical difference in the follow-up period between both groups.

progression in SBMA. A biomarker is an objectively measurable parameter that indicates the pathogenic process and potentially serves as a surrogate endpoint in a treatment trial. Candidates for biomarkers in neurodegenerative diseases include: motor functional scales; serological parameters; electrophysiological data; histopathological findings; and neuroimaging parameters. It is feasible to analyze combinations of biomarkers to monitor disease progression. There are an increasing number of biomarkers for Alzheimer's disease, but there is a paucity of biomarkers identified in other neurodegenerative diseases. In fact, identification of biomarkers has been hampered by small numbers of patients, slow disease progression, and lack of objective clinical measurements for SBMA. In the present study the 6MWD was significantly decreased in patients with SBMA compared with age-matched healthy subjects, and it correlated well with other scores that measure general motor function. This suggests that the test is capable of detecting motor impairment in SBMA patients. Furthermore, our longitudinal analysis showed that the 6MWD decreases by ≈10% per year in SBMA patients despite no detectable deterioration in the other motor functional parameters we examined. According to our sample size calculation, the number required for a clinical trial appears to be reduced to one-fifth by changing the endpoint from motor scores to 6MWD. For example, when the therapeutic effect is estimated to be 50%, a trial targeting ALSFRS-R needs 500 patients, but the size is diminished to 100 by adopting 6MWD as the endpoint (Table 4). Although it is a limitation of this study that the follow-up data for 11 out of 35 cases was not obtained, there was no statistical difference between the backgrounds of the followed subjects and the remaining cases. This suggests that the 24 patients who underwent the follow-up study represent the whole group. Because the interval between the onset of weakness and the need for a wheelchair has been reported to be ≈15 years, the observed rate of decrease in the 6MWD is likely reasonable.[5] Therefore, our findings also suggest that the 6MWT is an indicator of disease progression, which can be used as an outcome measurement in future clinical trials for SBMA. Our observation that the annual change in 6MWD is influenced by the disease severity might suggest the need for stratification in the design of clinical trials. Given that scrotal skin biopsy analysis is

Table 4. Sample size calculation.

	Estimated therapeutic effect (%)	
Outcome measure	50	70
6MWD	102	52
ALSFRS-R	508	259
Limb Norris Score	462	236
Norris Bulbar Score	2,650	1,352

Data are shown as the number of patients per group (P = 0.05, power = 0.8).
ALSFRS-R, ALS functional rating scale-revised.

a potent pathogenic marker of SBMA, the 6MWT might be used in combination with other biomarkers in order to determine response to therapeutics.[6]

In conclusion, our observations suggest that the 6MWT is a reliable biomarker to quantify exercise capacity in patients with neuromuscular disorders such as SBMA.

We thank Dr. Hiroshi Yatsuya for statistical advice. This work was supported by a Center-of-Excellence (COE) grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan, grants from the Ministry of Health, Labor and Welfare of Japan, a grant from Japan Intractable Diseases Research Foundation and the Program for Improvement of Research Environment for Young Researchers from Special Coordination Funds for Promoting Science and Technology (SCF) commissioned by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Journal of the Neurological Sciences 271 (2008) 68–74

Journal of the Neurological Sciences

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Prefrontal hypoperfusion and cognitive dysfunction correlates in spinocerebellar ataxia type 6

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Received 30 October 2007; received in revised form 18 March 2008; accepted 24 March 2008
Available online 7 May 2008

Abstract

Objective: The aim of this study is to evaluate the correlation between brain perfusion and cognitive dysfunction in spinocerebellar ataxia type 6 (SCA6) patients.
Methods: Thirteen genetically confirmed SCA6 patients and 21 age- and education-matched control subjects were subjected to single photon emission computed tomography (SPECT) and neuropsychological tests. Brain perfusion was examined with SPECT analysis, while general cognition, verbal and visual memory, attention, visuospatial ability, language, executive function, depression, and anxiety were examined with the neuropsychological tests.
Results: SCA6 patients showed prefrontal hypoperfusion, and impairments of visual memory, verbal fluency, and executive function compared to control subjects. These neuropsychological impairments in SCA6 patients were significantly correlated with a decrease in prefrontal perfusion. This relation was not correlated to other factors, such as age, education and severity of cerebellar ataxia, which are possible relevant factors associated with cognitive performance.
Conclusions: SCA6 patients have mild cognitive impairment, and correlating prefrontal hypoperfusion. These results indicate cognitive impairment in SCA6 patients resulting from prefrontal hypoperfusion.

Keywords: SPECT; Spinocerebellar ataxia type 6; Neuropsychological tests; Higher nervous activity; Cerebral blood flow; Cerebellum; Prefrontal cortex

1. Introduction

Spinocerebellar ataxia type 6 (SCA6) is an autosomal dominant cerebellar atrophy caused by an unstable CAG trinucleotide repeat expansion present in a gene on chromosome 19p13 that encodes the voltage-dependent α1A-subunit of the calcium channel CACNA1A [1]. This channel is highly expressed in cerebellar Purkinje cells and is of critical importance for the function and the development of cerebellar Purkinje cells.

Ataxia, gait disturbance, and dysarthria develop slowly in most SCA6 patients. Neurologic signs often associated with other spinocerebellar degeneration (SCD) disorders are seldom associated with SCA6, and so SCA6 is characterized as pure cerebellar ataxia [2].

Recently, there has been increasing evidence of the nonmotor role of the cerebellum [3,4], and clinical reports of patients with cerebellar lesions have implicated the cerebellum as having a role in cognitive functions [5,6]. Many of these clinical and imaging studies in cerebellar patients suffer from methodological shortcomings concerning patient

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0022-510X/$ - see front matter.
doi:10.1016/j.jns.2008.03.018

selection, such as the inclusion of patients with additional extracerebellar damage. Although regional cerebral blood flow (rCBF) and cognitive function in spinocerebellar ataxia patients have been examined before genetic analysis became available [7,8], these studies were controversial since they included many types of spinocerebellar ataxia with additional extracerebellar damage.

Neuropathologically, SCA6 is characterized by almost exclusive cerebellar involvement particularly selective loss of the cerebellar Purkinje cells [9]. Degeneration is mostly restricted to the cerebellum in SCA6 patients while cortical structures and basal ganglia are spared. SCA6 therefore represents an excellent model for investigating the cerebellar contribution to cognition. So far, however, only a few studies have examined the rCBF of SCA6 patients [10]. Globas et al. failed to provide clear evidence for cognitive deficits in SCA6 patients [11]. However, we recently revealed significant impaired visual memory and verbal fluency in genetically confirmed SCA6 patients [12], suggesting that SCA6 patients have prefrontal dysfunction. Previous studies have indicated hypoperfusion restricted to the cerebellum [10], however these results may be attributable to the small sample size of the SCA6 patients used. In previous studies, VOI analysis methods was used [10], and they may be not entirely objective because we could not examine the perfusion of whole brain by VOI analysis methods.

The aims of this study were to clarify the rCBF and to evaluate the relationships between rCBF and cognitive dysfunction in genetically confirmed SCA6 patients.

2. Methods

2.1. Subjects (Table 1)

Thirteen genetically confirmed SCA6 patients from thirteen families and 21 control subjects were enrolled in this study (Table 1). These 13 SCA6 patients were from the same pool of 18 patients who were examined in our previous report [12]. All were native Japanese speakers. Severity of ataxia was rated on the International Cooperative Ataxia Rating Scale (ICARS) [13]. Genomic DNA was extracted from the peripheral blood of the SCA6 patients using a conventional technique [14]. PCR amplification of the CAG repeat in the CACNA1A gene was performed using a fluorescein-labelled forward primer (5′-AGCCCCCTCAACATCTGGTA-3′) and a non-labelled reverse primer (5′-GACCCGCCTCTCCATCCT-3′). PCR conditions after a 3-min initial denaturation at 94 °C, were 35 cycles of 94 °C for 1 min, annealing at 64 °C for 1 min, and elongation at 72 °C for 1 min. Aliquots of PCR products were combined with loading dye and separated by electrophoresis with an autoread sequencer SQ-5500 (Hitachi Electronics Engineering, Tokyo, Japan). The size of the CAG repeat was analyzed on Fraglys software version 2.2 (Hitachi) by comparison with co-electrophoresed PCR standards with known repeat sizes. The CAG-repeat size of the PCR standard was determined by direct sequence using a sequence primer (5′-ACATCTGGTACCAGCACTCC-3′). No SCA6 patient included in this study had neurological signs and symptoms suggestive of other neurological or psychiatric diseases. Age- and education-matched paid volunteers were recruited as control subjects for neuropsychological tests and perfusion studies. Control subjects had no history of any neurologic or psychiatric disease that affected cognition. Written informed consent was received in advance from SCA6 patients and control subjects. The study was approved by the ethics committee of the Nagoya University Hospital.

Table 1
SPM results for group comparison between SCA6 patients and control subjects

Region	k_E	Coordinates			Z
		x	y	z	
		{mm}			
Cerebellum, brain stem	18,462	−6	−56	−12	Inf
		8	−58	−12	Inf
		−18	−68	−18	6.93
Left middle frontal gyrus, medial frontal gyrus	325	−28	8	42	3.16
		−8	0	50	2.71
		−24	0	40	2.50

SPM, statistical parametric mapping; SCA6, spinocerebellar ataxia type 6.

2.2. Neuropsychological tests

Based on our previous study [12], we selected neuropsychological tests on which SCA6 patients might have impairments. Each patient underwent a standard cognitive status assessment. All of the patient's neuropsychological tests were given on the same day. The Mini-Mental State Examination (MMSE) was used as a screen [15]. Visual memory was examined using the Visual Paired Associates Subtests 1 and 2 of the Revised Wechsler Memory Scale (WMS-R) [16]. Verbal memory was examined using the Logical Memory Subtests 1 and 2 of the WMS-R [16]. To evaluate verbal fluency as a measure of language function, subjects were asked to name as many items as possible within 1 min from a semantic category (animals) and from a phonemic category (Japanese nouns starting with the Japanese Kana character "Ka"). To evaluate executive function, the Rule Shift Cards test of the Behavioural Assessment of the Dysexecutive Syndrome (BADS) was used [17]. In trial 1 of this test, 21 spiral-bound non-court playing cards are turned over one by one, and the subject is asked to say "yes" to a red card and "no" to a black card. In trial 2, the subject is asked to say "yes" if the card is the same color as the previous one and "no" if it not. We statistically analyzed the relationships between results of the neuropsychological tests and their characteristics, including disease duration, degree of ataxia (ICARS), and CAG-repeat length. For the neuropsychological tests in which rapid speech was an important aspect of performance (e.g., the phonemic and

semantic fluency task), we analyzed the correlations to the dysarthria subscore of the ICARS.

2.3. Assessment of regional brain perfusion with SPECT

600 MBq of ^{99m}Tc-Ethylcysteine dimer (ECD) (Neurolite, Daiichi Radioisotope Laboratories, Ltd, Tokyo, Japan) was injected intravenously while the subjects were in a supine position with eyes closed in a quiet dimly lit room. SPECT scanning was carried out between 5 and 30 min after injection using a triple-head GCA 9300A gamma camera (Toshiba, Tokyo, Japan) equipped with low energy, super high resolution, fan-beam collimators. The data were acquired in a 128×128 matrix through a 120° rotation at an angle interval of 4°. The projection data were prefiltered through a Butterworth filter, and reconstructed using filtered backprojection with a Ramp filter. No attenuation correction was performed. The in-plane spatial resolution was 8 mm in full width at half maximum (FWHM). The final image slices were set up parallel to the orbitomeatal line and were obtained at an interval of 6.9 mm through the entire brain.

2.4. Data analysis

For the statistical analysis of the neuropsychological tests, SPSS version 11.0 for Windows (SPSS Japan, Tokyo, Japan) was used. The Shapiro–Wilk test was used to assess the normality of continuous variables. For comparison between the SCA6 group and the control group, we performed an unpaired t test for normally distributed data or a non-parametric Mann–Whitney U test for non-normally distributed data. Statistical significance was chosen at a P value of 0.05, with the correction for multiple comparisons using Holm–Sidak method. For correlation studies we used the Pearson product moment correlation test for normally distributed variables or the Spearman rank correlation test for non-normally distributed variables.

The SPECT data were analyzed using Statistical Parametric Mapping version 2 (SPM2; Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK) implemented in MATLAB version 6.5.1 (Math Works, Sherborn, MA, USA). In a pre-processing step, datasets were spatially normalized to a standard stereotactic three dimensional space and smoothed with an isotropic Gaussian kernel of FWHM 12 mm. All of the images resulting from the normalization procedure were visually acceptable. In the following analyses, proportional scaling was applied to adjust the mean whole brain activity to 50 ml/100 g/min to avoid inter-individual variation in global cerebral blood flow. The grey matter threshold was 0.8. The normalized images of the SCA6 patients and control subjects were compared with voxel by voxel t statistics. Confounding effects may arise from age or education differences, which could influence regional cerebral blood flow change in SCA6. Therefore, age and education were inserted as covariates in the statistical parametric mapping (SPM) analyses. In addition, we compared the images of the SCA6 patients to detect voxels in which the rCBF was significantly correlated with the scores of neuropsychological tests on which SCA6 patients showed impairment. Each value of the neuropsychological tests was used as a covariate of interest, and the values of the global rCBF, age, education and ICARS, which are the relevant factors associated with cognitive performance, were excluded as nuisance variables [18]. Regions were reported as significant if they contained voxels with a value of at least $P<0.01$, uncorrected for multiple comparisons, with cluster extent threshold (k_E)=100.

3. Results

3.1. Neuropsychological features

We assessed the neuropsychological features of 13 patients out of 18 patients who were examined in our previous study [12], since these patients could receive both neuropsychological tests and SPECT. The test results were similar to our previous report [12]. SCA6 patients did not differ significantly from controls with regard to MMSE, but showed significant cognitive impairment in several other tasks (Supplemental Table 1). Performance on the Visual Paired Associates Subtest 1 was significantly reduced in SCA6 patients compared with controls ($P=0.003$), while performance on the Visual Paired Associates Subtest 2 was not. Verbal fluency tasks in semantic and phonemic

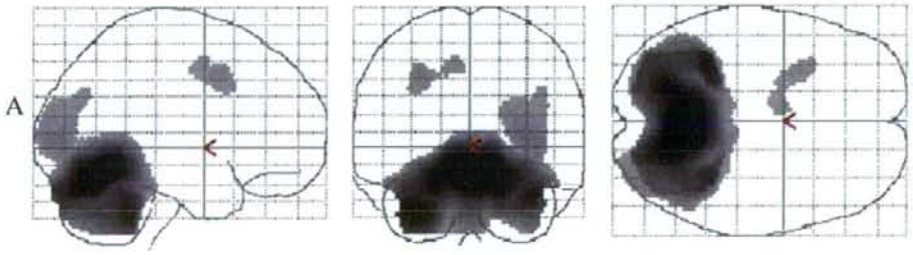

Fig. 1. SPM maps comparing brain perfusion between SCA6 patients and control subjects (uncorrected $P<0.01$). Sagittal (left column), coronal (middle column) and transverse (right column) views of the standard brain.

categories were remarkably impaired in SCA6 patients ($P=0.006$, $P=0.006$, respectively). The result of the Rule Shift Cards test tasks showed a tendency to be impaired in SCA6 patients compared with controls, although not significant. These neuropsychological impairments in SCA6 patients did not show any significant correlation to CAG-repeat length and disease duration, nor to ICARS dysarthria subscores. No significant differences were observed between SCA6 patients and control subjects in verbal memory function as tested using Logical Memory Subtests 1 and 2 of the WMS-R.

3.2. Correlation between rCBF and cognitive dysfunction in SCA6 patients

According to SPM analyses, SCA6 patients showed reduced brain perfusion in the left middle frontal and medial frontal cortices, as well as in the cerebellum and brain stem, compared to control subjects (uncorrected $P<0.01$; Fig. 1 and Table 1).

We examined the correlation between rCBF and cognitive dysfunction after age, education, and severity of cerebellar ataxia were excluded statistically (Fig. 2 and Table 2). There

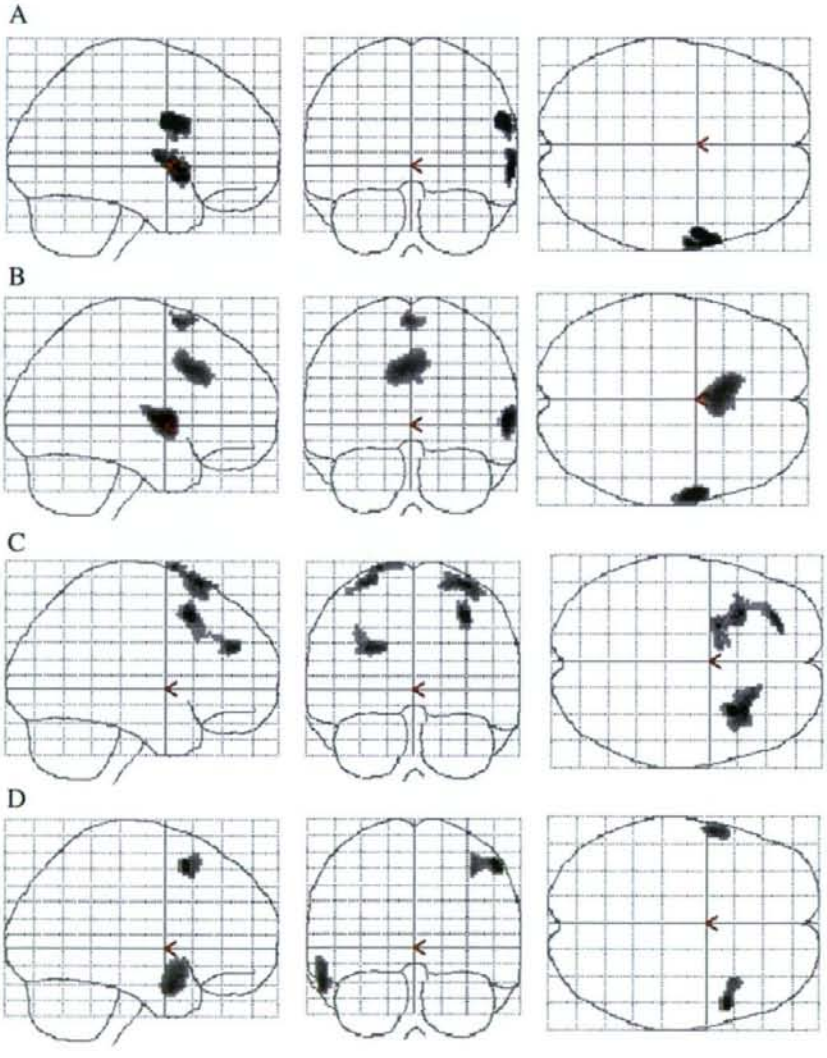

Fig. 2. SPM maps of the correlation between brain perfusion in SCA6 patients and cognitive performance (uncorrected $P<0.01$). Sagittal (left column), coronal (middle column) and transverse (right column) views of the standard brain. A: Visual Paired Associates 1 test, B: phonemic fluency test, C: semantic fluency test, D: Rule Shift Cards test.

Table 2
SPM results of correlations between the results of the neuropsychological tests and brain perfusion in SCA6 patients

Region	k_E	Coordinates			Z
		x	y	z	
		{mm}			
Perfusion positively correlated with the score of the Visual Paired Associates I					
Right inferior frontal gyrus	163	56	2	30	2.94
		60	10	28	2.79
Right superior temporal gyrus	143	62	8	−2	2.78
		64	−4	6	2.65
Perfusion positively correlated with the score of the phonemic fluency					
Right superior temporal gyrus	270	60	2	4	3.91
Cingulate gyrus	521	−4	14	38	3.11
		4	12	40	2.91
		−10	24	34	2.91
Medial frontal gyrus	115	−2	8	68	2.96
		−2	18	68	2.69
		8	14	66	2.46
Perfusion positively correlated with the score of the semantic fluency					
Right middle frontal gyrus	110	32	16	48	3.80
		30	24	40	2.34
Right superior frontal gyrus	168	32	20	68	3.39
		22	28	66	2.82
		22	26	74	2.44
Left superior frontal gyrus	113	−22	6	78	3.19
		−32	20	68	3.14
		−24	16	72	2.88
Left middle frontal gyrus	103	−30	40	26	3.05
		−36	22	36	2.49
		−34	30	34	2.45
Perfusion positively correlated with the score of the Rule Shift Cards test					
Right superior frontal gyrus	130	52	12	52	3.67
		36	18	48	2.73
		38	18	56	2.70
Left superior temporal gyrus	126	−58	6	−20	2.98
		−60	10	−10	2.73

SPM, statistical parametric mapping; SCA6, spinocerebellar ataxia type 6.

was a positive correlation between the score of the Visual Paired Associates I test and the perfusion in the right inferior frontal and right superior temporal gyri in SCA6 patients. In SCA6 patients, there was a positive correlation between the phonemic fluency score and the perfusion in the medial frontal and superior temporal gyri, between the semantic fluency score and the perfusion in the bilateral superior and middle frontal gyri, and between the Rule Shift Cards test score and the perfusion in the right middle frontal and superior temporal gyri.

4. Discussion

In this study, we demonstrate that SCA6 patients have a reduction of rCBF in not only the cerebellum but also prefrontal cortices. In our previous study, we documented that visual memory, verbal fluency, and executive function were significantly impaired in SCA6 patients [12]. In the present study, we show that the rCBF in prefrontal cortices is significantly correlated to these neuropsychological impairments in SCA6 patients.

rCBF had been examined in patients with various types of spinocerebellar ataxia before we could confirm our diagnosis of spinocerebellar ataxias genetically [7,8]. However several types of diseases might be included in these studies, and the results were not consistent. Recently, one study examined rCBFs in genetically confirmed SCA6 patients and described only cerebellar hypoperfusion [10]. These results agree with results of previous neuropathologic studies showing that lesions of SCA6 are restricted to the cerebellum, where abundant expression of P/Q-type calcium channels has been found [19]. Magnetic resonance imaging analysis of SCA6 patients demonstrated no abnormalities in the central nervous system except for cerebellar atrophy [20]. However, one study showed that crossed cerebello-cerebral diaschisis (CCCD) was derived from the functional deactivation of the cerebello-ponto-thalamo-cerebral pathways [21]. Unilateral impairment of the cerebellum would lead to reduced radioisotope uptake in the contralateral cerebral hemisphere, and CCCD has been established by PET and SPECT studies in various cerebellar diseases [8]. Therefore, CCCD might be also found in SCA6 patients. In previous studies, a statistically significant difference of prefrontal perfusion could not be established between SCA6 patients and controls, which may be due to the small sample size of patients [10]. Recently, new SPECT analysis techniques such as SPM have been developed that allow brain functional images to be studied more easily and accurately than before. It is entirely automated and objective, and completely overcomes the disadvantage of earlier VOI analysis methods. Although imaging studies using SPM have been performed on various diseases recently, there has been only one study examining SCA6 patients by SPM, revealing the reduction of metabolism in frontal and prefrontal cortices [22]. In the present study, we examined the perfusion of whole brain in SCA6 patients using SPM, and revealed hypoperfusion in prefrontal cortices as well as cerebellum. We speculate that the mechanism of rCBF reduction in prefrontal cortices of SCA6 patients is the functional deactivation of the cerebello-ponto-thalamo-cerebral pathways.

In this study, we reveal visual memory deficits, impairments of verbal fluency, and executive dysfunction in SCA6 patients, which are similar to those described in patients with spinocerebellar ataxia type 1 [23], type 2 [24,25], and Machado–Joseph disease [26,27]. Cognitive dysfunction in these diseases is considered to be derived from the disruption of the cortico-cerebellar loop [23,24,26,27]. However, these diseases have extracerebellar involvement with degeneration of frontal lobes, thalamus, brainstem or basal ganglia, [28,29]. Cognitive impairment is therefore likely to result from additional extracerebellar damage as well as cerebellar degeneration itself in these disorders. Because lesions in SCA6 patients are restricted to the cerebellum, we suggest that the cognitive dysfunction in SCA6 patients derives from

the disruption of the cortico-cerebellar loop. There are a few studies that address cognitive function in SCA6 patients, and a previous study could not disclose a significant impairment of attention, working memory, verbal and visuospatial memory, or fronto-executive functions [11]. However, the lack of statistical significance in their study may be due to an insufficient sample size of SCA6 patients or to other methodological issues such as different neuropsychological tests.

We found correlations between the score on the Visual Paired Associates 1 test and the perfusion in the right inferior frontal and right superior temporal gyri, between the score on the phonemic fluency test and the perfusion in the medial frontal and superior temporal gyri, between the score on the semantic fluency test and the perfusion in the bilateral superior and middle frontal gyri, and between the score on the Rule Shift Cards test and the perfusion in the right middle frontal and superior temporal gyri in SCA6 patients. These lesions were mostly consistent with the prefrontal hypoperfusion in SCA6 patients. Previous studies demonstrated that frontal lobe function is associated with visual memory task [30,31]. In addition, impairments of verbal fluency have been considered to reflect frontal lobe damage[32]. The Rule Shift Cards test is used for measuring the executive dysfunction, including frontal lobe dysfunction. Taken together, the cognitive dysfunctions seen in SCA6 patients may suggest prefrontal involvement, although the cerebral cortex is well preserved [9].

In summary, SCA6 patients have mild cognitive impairment, and prefrontal hypoperfusion, and these results are related to each other. These data indicate that cognitive impairment in SCA6 patients may result from prefrontal hypoperfusion.

Acknowledgement

This study was supported by grants from the Ministry of Health, Labour, and Welfare of Japan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jns.2008.03.018.

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Journal of the Neurological Sciences 271 (2008) 40–46

Journal of the Neurological Sciences

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Fractional anisotropy values detect pyramidal tract involvement in multiple system atrophy

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Received 27 December 2007; received in revised form 8 March 2008; accepted 14 March 2008
Available online 23 May 2008

Abstract

Objective: Pathological studies have shown remarkable pyramidal tract involvement in multiple system atrophy (MSA), while clinical pyramidal signs are relatively rare. We investigated the fractional anisotropy (FA) values to assess the degree of pyramidal tract involvement in MSA, in comparison with amyotrophic lateral sclerosis (ALS) and controls. Furthermore, we compared FA values between MSA patients with or without clinical pyramidal signs and controls, and between MSA patients with or without positive conventional MRI findings and controls.

Methods: We evaluated FA values in the internal capsule, corona radiate and whole pyramidal tract using visualized tractography of 65 subjects (20 probable MSA patients, 28 age-matched ALS patients, and 17 age-matched healthy controls) using a 3.0T magnetic resonance system.

Results: The FA values in the internal capsule, corona radiate, and whole pyramidal tract were significantly lower in MSA patients than in controls and were at a level similar to those of ALS patients. In addition, low FA values were prominent in MSA patients, even in those with short duration of illness, lacking precentral gyrus hyperintensity in FLAIR images, and without pyramidal signs.

Conclusion: FA values could identify pyramidal tract degeneration even in patients with early phase MSA and those without clinical pyramidal signs or abnormal MRI findings. More extensive degeneration of the pyramidal tract occurs in MSA than so far believed.

Keywords: Multiple system atrophy; Pyramidal tract involvement; Fractional anisotropy values; Amyotrophic lateral sclerosis; Small-sized neuron; Tractography

1. Introduction

Multiple system atrophy (MSA) is a sporadic, adult-onset neurodegenerative disease [1,2]. The nigrostriatal, olivopontocerebellar, and autonomic systems are often and prominently impaired; the clinical signs of the degeneration of these systems are major diagnostic criteria for MSA [3]. Although pathological studies have shown widespread pyramidal tract involvement in MSA, [4–6] extensor plantar responses with hyperreflexia occur in about 30–50% of patients [7,8]. Thus, pyramidal signs are thought to be less important in diagnosis than the symptoms seen in the other systems. In addition, electrophysiological or magnetic measures to monitor central motor conduction have not been able to detect pyramidal tract degeneration in MSA [9–11].

Fractional anisotropy (FA) values are quantitative parameters of magnetic resonance imaging (MRI) [12], and have been used to evaluate the degree of tissue degenerations in various disorders. FA values measure the degree of anisotropy of the diffusing water along different axes of the image, and decreasing FA values represent tissue degeneration in normal

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doi:10.1016/j.jns.2008.03.013

aging [13] or various diseases [14–16]. As for the evaluation of pyramidal tract degeneration using FA values, amyotrophic lateral sclerosis (ALS), in which the pyramidal tract is prominently involved, has been well studied, showing low FA values in some regions of pyramidal tract as a result of neuronal degeneration [14,17].

Recent studies have shown that FA values are reduced in the CNS regions in multiple system atrophy predominated in cerebellar ataxia (MSA-C) [18]. FA values have also been used to discriminate multiple system atrophy predominated in parkinsonism (MSA-P) from Parkinson's disease (PD) [19,20]. However, FA values have not been studied for their usefulness detecting pyramidal tract degeneration in MSA. T1 spine-echo imaging, with an additional magnetization transfer contrast pulse (T1 SE/MCT) sequence has recently shown to detect the pyramidal tract degeneration in MSA [21]. However, the extent and features of pyramidal tract degeneration in MSA have not been well evaluated by neuroimaging including FA values.

The aim of the present study is to examine the pyramidal tract involvement in MSA using FA values, to correlate these findings to clinical signs and MRI findings, and further compare to the extent of those seen in ALS.

2. Methods

We studied 65 subjects in total; 20 patients with probable MSA (10 MSA-C; 10 MSA-P), 28 age- and gender-matched patients with ALS, and 17 age- and gender-matched healthy volunteers (Table 1). This study was approved by the ethics committee of the Nagoya University Graduate School of Medicine, and informed consent was established before participation to the study. Clinical diagnoses of MSA and ALS were established by consensus diagnostic criteria [3,22]. All MSA patients fulfilled the criteria for probable MSA. All ALS patients fulfilled the clinically definite or clinically probable-laboratory supported revised ALS criteria of El Escrial. Healthy controls underwent the same MRI examination as the MSA and ALS patients.

There were no differences in mean age or gender distribution between the MSA, ALS or control populations. There was a significant variation in time from initial symptoms to MRI evaluation among the MSA patients (4±2 years, range: 1 to 10 years) and ALS patients (2±1 years, range: 1 to 5 years), including both MSA and ALS patients in relatively early disease stages in this study.

Table 1
Patients data

	Number of cases	Age (years)	Female/ male	Duration (years)	With/without the pyramidal signs
MSA	20	61±9	8/12	4±2	10/10
MSA-P	10	63±11	4/6	4±3	5/5
MSA-C	10	58±7	4/6	4±2	5/5
ALS	28	62±12	9/19	2±1	28/0
Control	17	61±11	12/5		

MSA; multiple system atrophy, MSA-P; multiple system atrophy predominated in parkinsonism.
MSA-C; multiple system atrophy predominated in cerebellar ataxia, ALS; amyotrophic lateral sclerosis.

Information on the presence of the pyramidal signs was obtained from the neurological examinations or from patient medical records. We defined patients as having pyramidal signs when they showed increased tendon reflexes or extensor plantar responses. Ten MSA patients showed pyramidal signs (five MSA-C patients, five MSA-P patients), the other ten patients did not (five MSA-C patients, five MSA-P patients), and all ALS patients showed pyramidal signs, although the extent and combination of signs varied.

2.1. MRI protocol

All scanning was carried out with a 3.0T magnetic resonance scanner (Trio, Siemens, Erlangen, Germany), using a receive-only 8-channel phased-array head coil. Diffusion weighted imaging (DWI) was obtained with optimal methods [23] using a Stejskal–Tanner sequence with single-shot spin-echo-type echo-planar imaging, a flip angle of 90°, and a repetition time of 7700 ms. Echo times corresponding to respective b-factors were 75 ms for 700 s/mm^2. Echo spacing was 0.79 ms, and the matrix size was 128×128 with a readout bandwidth of 1562 Hz/pixel. Sixty axial slices, 2 mm thick with a 0.6-mm interslice gap, were used to image the entire brain with a 23-cm^2 square field of view.

A motion-probing gradient (MPG) was applied in 6 orientations after acquisition of b=700 images. The 128×128 data matrix was zero-fill interpolated to 256×256. An acceleration factor of two was applied using the parallel imaging technique, generalized autocalibrating partially parallel acquisitions (GRAPPA), [24] which is an extension of the simultaneous acquisition of spatial harmonics technique. Eddy current-related geometric distortions were not prominent between the images of each MPG directions; thus, distortion correction post-processing was not applied.

2.2. Data analyses

FA values and tractography were obtained using public domain software dTV II for DWI analysis developed by the Imaging Computing and Analysis Laboratory, Department of Radiology, University of Tokyo Hospital, Japan, and made available at http://www.ut-radiology.umin.jp/people/masutani/dTV.htm.

To set regions of interest (ROIs), we visualized the pyramidal tract using tractography. In this study, we determined the pyramidal tract as visualized fibers by tractography that the seed area was defined as the cerebral peduncle and the target area as the precentral gyrus on T2-weighted axial images (b=0) (Fig. 1A). The ROIs in the internal capsule and corona radiate were placed within closed curves drawn on visualized pyramidal tracts (Fig. 1B, C). In addition, we set ROIs on

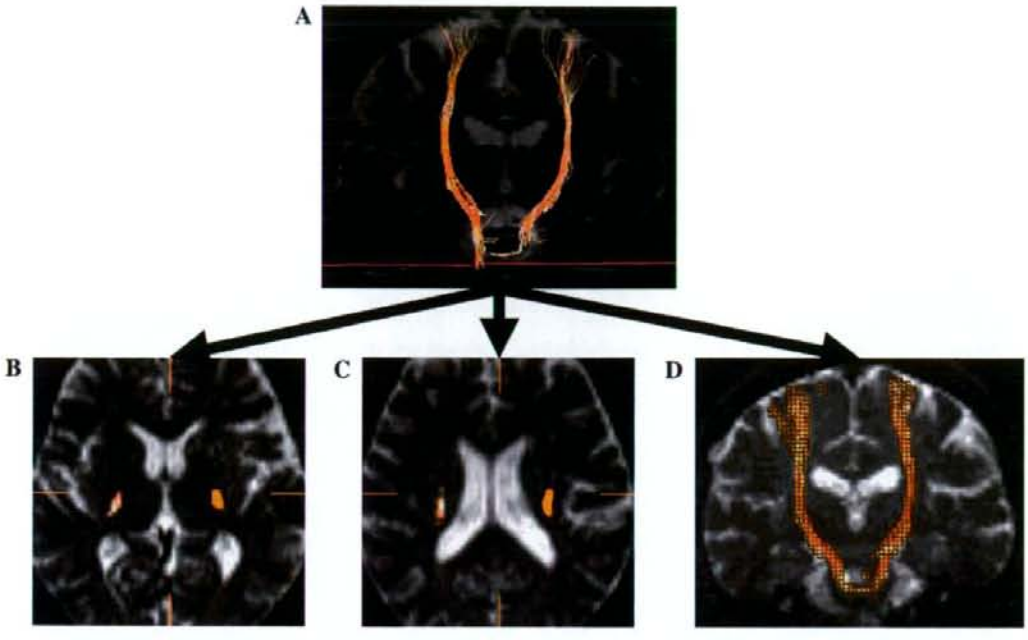

Fig. 1. Regions of interest. Visualizing the pyramidal tract using tractography (A). Regions of interest in the internal capsule (B), corona radiate (C) and whole pyramidal tract (D).

whole pyramidal tract visualized by tractography (Fig. 1D). Regional FA values were calculated for each region of interest. Mean FA values were adapted as representative indices of FA values.

In addition, the presence or absence of precentral gyrus hyperintensity on fluid attenuated inversion recovery (FLAIR) images was determined [21].

Statistical analyses were performed using SPSS 11.0 for Windows (SPSS Inc, USA). The Kruskal–Wallis test was used for comparisons of FA values among MSA and ALS patients, and controls, as well as MSA patients with and without pyramidal signs. The significance level was set at $p<0.05$.

3. Results

3.1. FA values in the internal capsule, corona radiate, and whole pyramidal tract

The tractography of the pyramidal tract was not visualized in one MSA-C and two ALS patients. The MSA-C patient's disease had been ongoing for 10 years and the advanced stage requiring confinement to bed. There was nothing particularly distinctive in the two ALS patients. As a result, we evaluated 19 MSA and 26 ALS patients. We adjusted the FA threshold to visualize tractography clearly. FA values in these three patients were too low to allow the reconstruction of the tractography.

FA values in all three regions of the MSA or ALS patients' brains were significantly lower ($p<0.05$–0.001) than those in controls (Fig. 2A–C). FA values were indistinguishable between MSA and ALS patients in the internal capsule and the corona radiate (Fig. 2A–C), however, FA values in the whole pyramidal tract were significantly lower in MSA patients than ALS patients. With respect to MSA phenotypes, FA values in the internal capsule and whole pyramidal tract were significantly lower ($p<0.01$–0.001) to a similar extent for both MSA-P and MSA-C patients (Fig. 2D, F), while those in the corona radiate tended to be lower in MSA-P and MSA-C patients, although the difference was not statistically significant (Fig. 2E). The FA values in the whole pyramidal tract were significantly lower ($p<0.05$–0.01) in MSA-C and MSA-P patients than ALS patients (Fig. 2F). The FA values in all three regions were similarly decreased in both MSA-C and MSA-P patients.

3.2. Correlation of FA values to precentral gyrus hyperintensity on FLAIR images and clinical pyramidal signs

Six MSA patients (30%) showed the precentral gyrus hyperintensity on FLAIR images, which is considered to be cortical pyramidal neuron involvement [21]. MSA patients both with or without precentral gyrus hyperintensity showed lower FA values in all three areas than seen in controls (Fig. 3A–C). The FA values in the internal capsule and whole pyramidal tract were significantly lower in MSA patients than in controls independent of those with or without precentral gyrus hyperintensity, while those in the corona radiate were

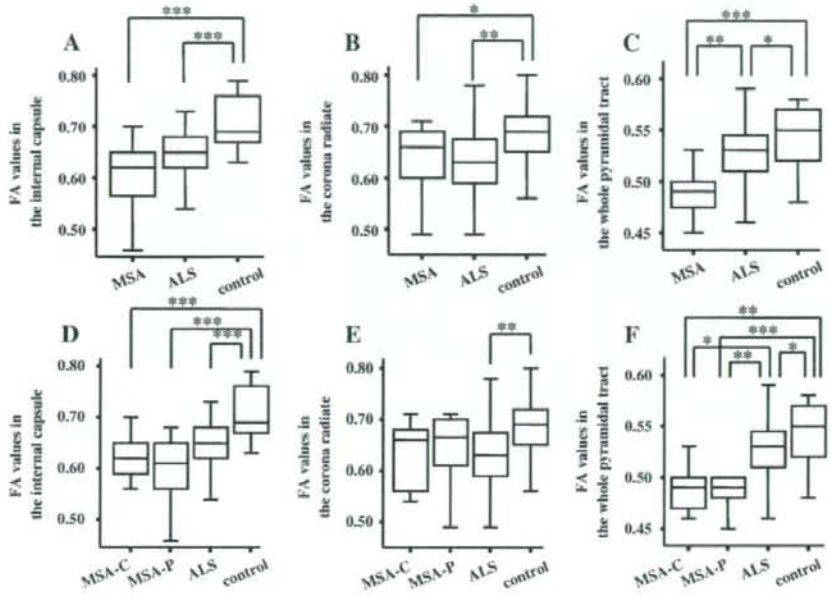

Fig. 2. FA values in the internal capsule, corona radiate, and whole pyramidal tract. FA values in MSA patients, ALS patients and controls or in MSA-C, MSA-P and ALS patients are shown in the internal capsule (A/D), corona radiate (B/E), and whole pyramidal tract (C/F). ***: $p<0.001$, **: $p<0.01$, *: $p<0.05$.

significantly lower in only the MSA patients with precentral gyrus hyperintensity. In addition, FA values in all three regions of interest were more decreased in MSA patients with precentral gyrus hyperintensity than those without it. Especially, FA values with precentral gyrus hyperintensity in the internal capsule were significantly lower than those without it. However, there were no significant differences between FA values with and without precentral gyrus hyperintensity in the corona radiate and whole pyramidal tract. These observations suggested that the FA values were likely decreased in almost all three anatomical regions even in the MSA patients without central gyrus hyperintensity.

We further examined the correlation between FA values and pyramidal signs in MSA patients. The MSA patients, both with or without pyramidal signs, showed lower FA values than controls in all three areas (Fig. 4A–C). FA values in the internal capsule and whole pyramidal tract were significantly lower in MSA patients with or without pyramidal signs than seen in controls, while FA values in the corona radiate were significantly lower in MSA patients

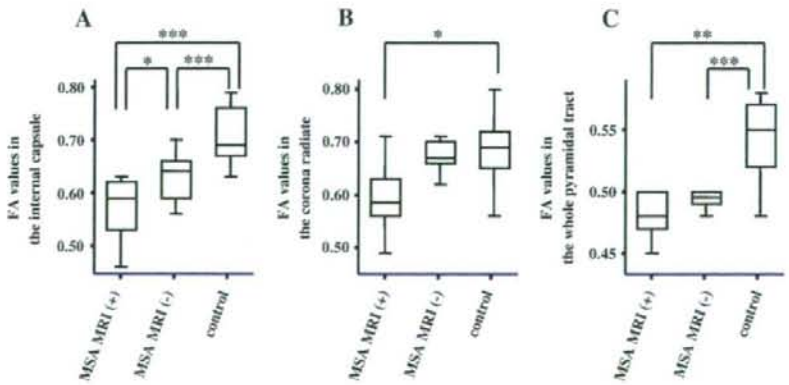

Fig. 3. Correlation between FA values and precentral gyrus hyperintensity on FLAIR images. FA values in MSA patients with or without precentral gyrus hyperintensity, and controls are shown for the internal capsule (A), corona radiate (B), and whole pyramidal tract (C). MSA MRI (+): MSA with precentral gyrus hyperintensity. MSA MRI (−): MSA without precentral gyrus hyperintensity. ***: $p<0.001$, **: $p<0.01$, *: $p<0.05$.