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

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

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

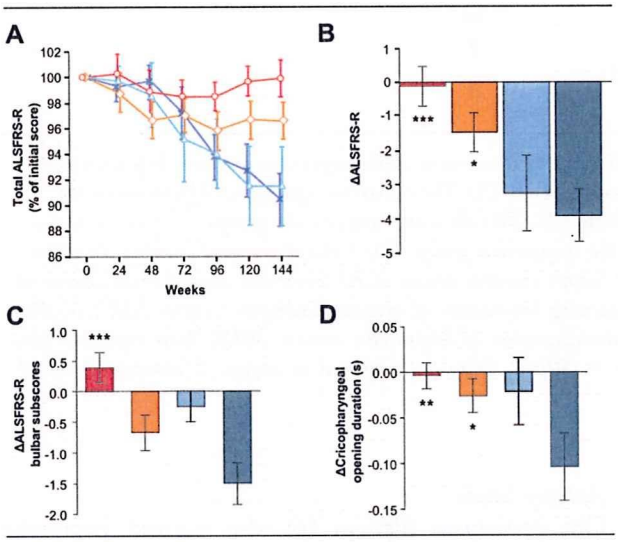

*Fig 3. Efficacy results of the 96-week follow-up trial. (A, B) Changes in the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) scores showed treatment duration–dependent improvements in the leuprorelin-treated groups. (C, D) The ALSFRS-R bulbar subscores (C) and videofluorography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

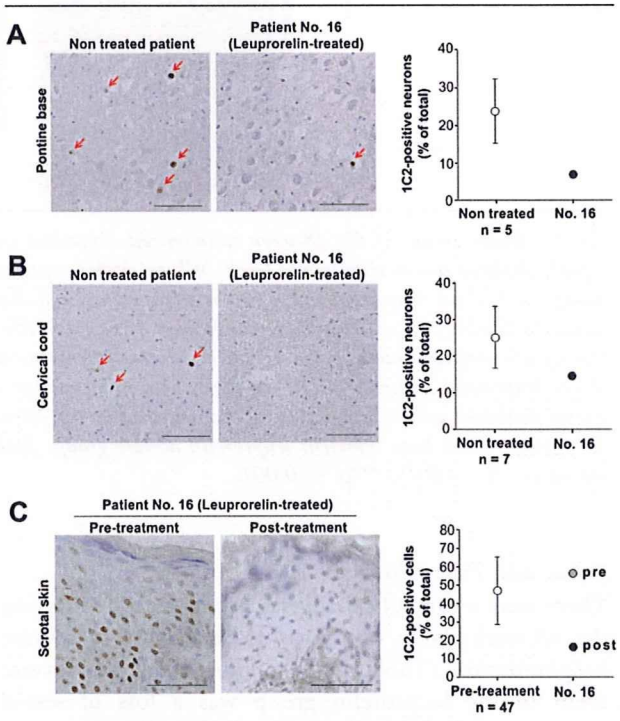

Fig 4. Effects of leuprorelin acetate on nuclear accumulation of mutant androgen receptor (AR). (A, B) Accumulation of mutant AR in neurons was remarkable both in the pontine base and in the spinal anterior horn of all the control, non-treated autopsied cases, but the number of 1C2-positive neurons was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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

H. Watanabe, MD
M. Hirayama, MD
A. Noda, PhD
M. Ito, MD
N. Atsuta, MD
J. Senda, MD
T. Kaga, MD
A. Yamada, MD
M. Katsuno, MD
T. Niwa, MD
F. Tanaka, MD
G. Sobue, MD

Address correspondence and reprint requests to Dr. Gen Sobue, Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan
sobueg@med.nagoya-u.ac.jp

ABSTRACT

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

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

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

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

GLOSSARY

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

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

From the Departments of Neurology (H.W., M.H., M.I., N.A., J.S., T.K., M.K., F.T., G.S.) and Cardiology (A.Y.), Nagoya University Graduate School of Medicine; and Departments of Medical Technology (A.N.) and Clinical Preventive Medicine (T.N.), Nagoya University School of Health Sciences, Japan.

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

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

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

All patients were assessed by an echocardiography GE VIVID 7 machine (GE Medical Systems, Milwaukee, WI) with two independent observers (A.N. and A.Y.) who were blinded to the clinical information. Mitral, aortic, and tricuspid valves were recorded from all possible views with the zoom function. In addition, a stethoscope examination was performed before echocardiography by A.N. and A.Y. Semiquantitative and quantitative measurements for quantification of regurgitant valvular diseases from the continuous wave, pulsed wave, and color Doppler examinations were assessed. Tenting distance and tenting area of the mitral valve were also evaluated as quantitative data.[7,8,10,15] We quantified regurgitant lesions by integration of all semiquantitative and quantitative measurements, and a final score was given as follows: absent, 0; trace, 1; mild, 2; moderate, 3; severe, 4.[27] A composite regurgitation score was calculated by adding the scores for aortic regurgitation (AR), mitral regurgitation (MR), and tricuspid regurgitation (TR).[7,13] The proportion of patients with any regurgitation grade from 3 to 4 was also assessed.[7] We derived the systolic pulmonary artery pressures from the TR jet, adding 10 mm Hg to the maximum gradient of the TR jet or 5 mm Hg if the vena cava inferior diameter was less than 10 mm with complete respiratory collapse and 15 mm Hg if the vena cava inferior was greater than 20 mm without respiratory variation. Left ventricular end-diastolic and end-systolic dimensions were measured, and the left ventricular ejection fraction was calculated by the Teichholz method. All patients with PD were investigated for both the diameter and flow of the hepatic vein and inferior vena cava using ultrasonography. In addition, a chest X-ray was also performed if necessary.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

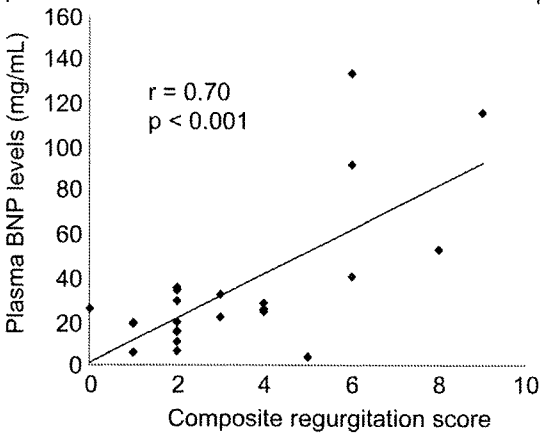
* The frequency of any grade 3 to 4 regurgitation was statistically significant between the ergot group and the non-ergot group ($p < 0.0001$) as well the ergot group and the control group ($p < 0.001$).

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

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

BNP = B-type natriuretic peptide.

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



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

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

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

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

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

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

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

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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.

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

YU TAKEUCHI, MD,[1] MASAHISA KATSUNO, MD, PhD,[1,2] HARUHIKO BANNO, MD, PhD,[1] KEISUKE SUZUKI, MD, PhD,[1] MOTOSHI KAWASHIMA, MD,[1] NAOKI ATSUTA, MD, PhD,[1] MIZUKI ITO, MD, PhD,[1] HIROHISA WATANABE, MD, PhD,[1] FUMIAKI TANAKA, MD, PhD,[1] and GEN SOBUE, MD, PhD[1]

[1] Department of Neurology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
[2] Institute for Advanced Research, Nagoya University, Nagoya, Japan

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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

Correspondence to: G. Sobue; e-mail: sobueg@med.nagoya-u.ac.jp or M. Katsuno; e-mail: ka2no@med.nagoya-u.ac.jp

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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.

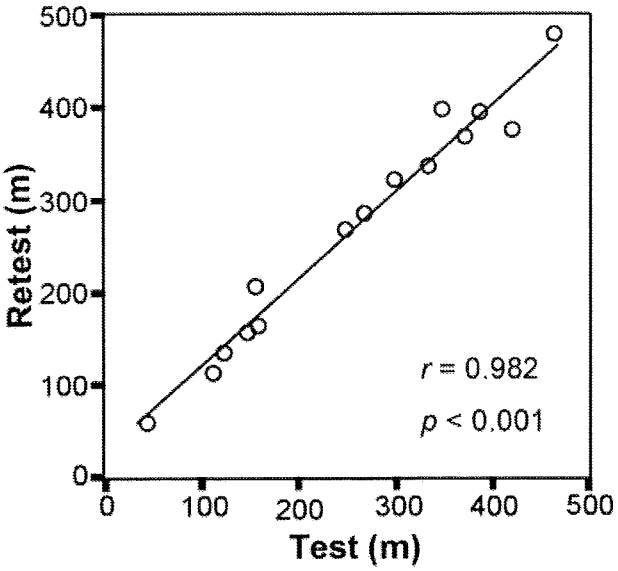

FIGURE 1. Test–retest analysis of the distance walked within 6 min (6MWD). Two sets of 6-min walk tests were carried out within 60 days in 15 SBMA patients who did not know the result of their previous test.

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.

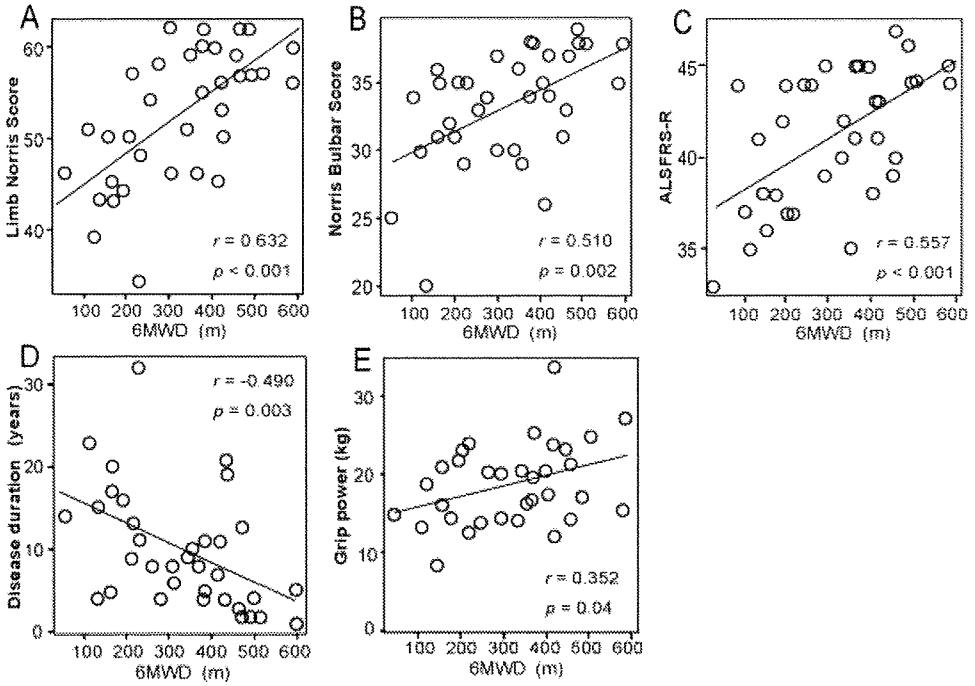

FIGURE 2. Correlation between the 6MWD and other measurements of motor function. **A–C:** The correlation between the 6MWD and general motor function. There was a significant correlation between the 6MWD and motor functional scales such as the Limb Norris Score **(A)**, the Norris Bulbar Score **(B)**, 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

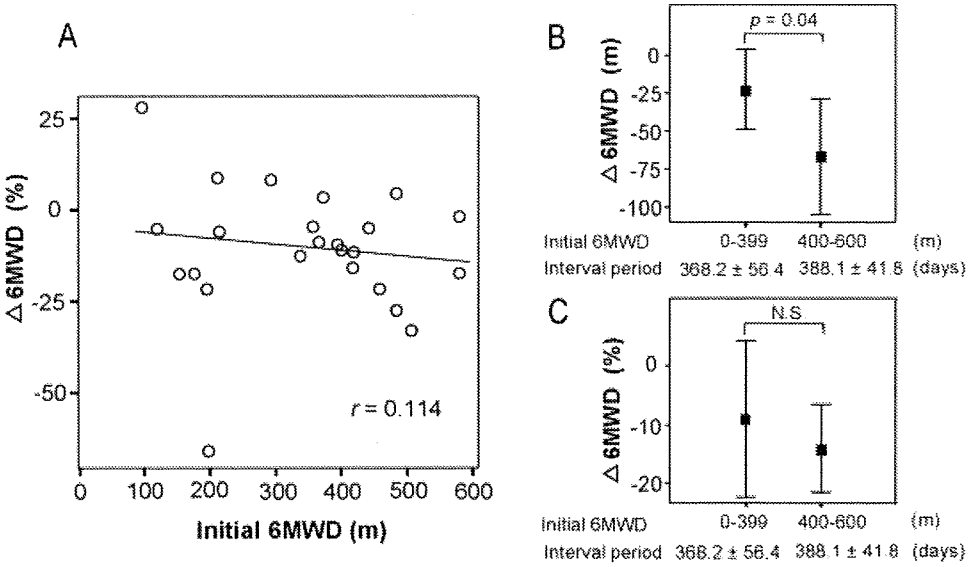

FIGURE 3. The relationship between the change in the 6MWD and the distance in the initial test. **A:** The decline in the 6MWD did not correlate with the result of the initial evaluation. **B,C:** The decline in the 6MWD for each of the severity groups (**B**, actual change; **C**, % change). There was 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.

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Prefrontal hypoperfusion and cognitive dysfunction correlates in spinocerebellar ataxia type 6

Y. Kawai [a], M. Suenaga [a], H. Watanabe [a], M. Ito [a], K. Kato [b], T. Kato [c], K. Ito [c], F. Tanaka [a], G. Sobue [a,*]

[a] *Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan*
[b] *Department of Radiology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan*
[c] *Department of Radiology, National Hospital for Geriatric Medicine, Aichi, Japan*

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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

* Corresponding author. Department of Neurology, Nagoya University Graduate School of Medicine, 65 Tsurumai-Cho, Showa-Ku, Nagoya 466-8550, Japan. Tel.: +81 52 744 2385; fax: +81 52 744 2384.
E-mail address: sobueg@med.nagoya-u.ac.jp (G. Sobue).

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