Table 1
Laboratory findings and clinical features

Patient age/sex	Dry eye/ dry mouth	Positive findings of Sjögren's syndrome	Initial symptom	Progression	Motor involvement		Sensory involvement					Autonomic involvement
					Weakness	Atrophy	Distribution	Superficial[a]	deep[b]	Spontaneous pain	Characteristics of pain	
1 67/M	-/-	SS-A, B lip biopsy	Pain	Chronic	-	-	L[c] (distal) T[c] (middle portion)	+	-	+++[d]	Aching hyperalgesia	1.2.3.4.5.6.
2 72/F	+/+	Lip biopsy	Pain	Chronic	-	-	L[c] (distal) H[c] (left side back)	+	-	+++[d]	Tingling hyperalgesia	1.2.4
3 54/M	+/+	SS-B lip biopsy	Sensory disturbance	Subacute	-	-	Right hand (radial) L[c] (dital) T[c] (upper portion)	+	-	+++[d]	Tingling static allodynia hyperalgesia	-
4 57/F	+/+	SS-A lip biopsy	Pain	Subacute	-	-	L[c] (distal)	-	-	+/+	Aching hyperalgesia	2.4
5 59/F	+/-	SS-A lip biopsy	Pain	Chronic	+	-	F[c] (left side) L[c] (upper) L[c] (right>left)	+	+	+++[d]	Tingling hyperalgesia	2.3.4

1, Abnormal pupils; 2, Hypohidrosis; 3, Orthostatic hypotension; 4, Constipation; 5, Urinary disturbance; 6, Decreased uptake of ^{123}I-MIBG.
[a] Superficial: reduction of superficial sensation including light touch and pinprick perception and temperature sensation.
[b] Deep: reduction of deep sensation including vibration and joint position.
[c] F, Face; H, Head; L, Limb; T, Trunk.
[d] 'Á(Á(, moderate pain; 'Á(Á(Á(, severe pain.

normal levels in these patients. Prior to treatment, all patients underwent neurological examination, blood studies, CSF studies, nerve conduction studies (NCS), and sural nerve biopsy. Profiles of the patients are summarized in Tables 1, 2, and 3. The group of patients included two men and three women, ranging from 54 to 72 years old. In all patients, the initial symptom of neuropathy was paraesthesia or painful peripheral dysaesthesia in the distal portion of the extremities.

Patient 1, a 67 year old man, was diagnosed as Sjögren's syndrome 16 years ago, had suffered painful dysaesthesia and numbness in the feet for 10 years, which spread to the proximal portion of the legs and arms. Neurological examination revealed a reduction in superficial sensation, including light touch/pinprick perception and temperature sensation; painful dysaesthesias were elicited over the middle portion of the trunk and the four extremities. The pain experienced in this patient's hands was so intense that he could not extend his fingers or touch objects. The pain in his feet almost precluded ambulation. *Patient 2*, a 72 year old woman had experienced painful dysaesthesia and numbness in the legs and hands for 3 years. Neurological examination revealed reduced superficial sensation along with painful dysaesthesias in the distal portion of the four extremities. *Patient 3*, a 54 year old man, had experienced pain in all four extremities and the head for 4 years. Neurological examination revealed no reduction in superficial sensation. Hyperalgesia was evident over the left side of the back of the head, the upper portion of the trunk, and the radial side of the right hand and feet. The patient needed to wear gloves to protect himself from the hand pain during his normal daily life. Sometimes, the patient also experienced difficulty walking as a direct result of pain. *Patient 4*, a 57 year old woman had suffered pain in her left foot for 1 year. Neurological examination revealed a reduction in superficial sensation; painful dysaesthesias were elicited over distal parts of the four extremities. *Patient 5*, a 59 year old woman had suffered spontaneous pain for 20 years along with painful dysaesthesia and numbness in all four extremities, but predominantly on the right side. Neurological examination revealed reduced superficial sensation. Painful dysaesthesias were elicited over the left-side cheek, the radial side of the upper extremities, and the lower extremities, predominantly on the right side. The pain in this patient's legs almost precluded ambulation.

Fluctuation in the intensity of pain was seen, to some extent, in all patients. Asymmetric pain symptoms and sensory impairments were seen in three patients (patients 3, 4, and 5). Although deep sensation, such as joint position and vibration, was mildly impaired in the distal portion of the extremities in one patient (patient 5), this was not accompanied by sensory ataxia, pseudoathetosis in the hand, or a

Table 2
Nerve conduction study

Patient	Median nerve					Tibial nerve			Sural nerve	
	MCV (m/s)	DL (ms)	CMAP (mV)	SCV (m/s)	SNAP (μV)	MCV (m/s)	DL (ms)	CMAP (mV)	SCV (m/s)	SNAP (μV)
1	56	3.4	5.4	48	5.2	42	4.2	7.1	47	3.9
2	55	3.1	4.5	64	23	32	4.1	5.7	43	17
3	55	3.1	12.6	63	25.1	48	3.9	15.8	51	27.2
4	54	2.9	10.3	58	41.6	40	4.6	10.5	60	15.5
5	55	2.7	6.1	62	25.2	42	3.4	15.9	48	11.5
Controls	57.6±3.8	3.4±0.4	8.2±2.9	56.3±5.3	28.0±11.5	46.0±3.8	4.0±0.6	11.8±3.5	49.2±4.8	16.8±7.8

Control values were obtained in 171 normal volunteers for the median nerve, 161 for the ulnar nerve, and 163 for the sural nerve [11].
MCV = motor nerve conduction velocity; DL = distal latency; CMAP = compound muscle action potential.
SCV = sensory nerve conduction velocity; SNAP = sensory nerve action potential.

Table 3
Pathological findings in the sural nerve

Patient	Myelinated fiber density (no/mm^2)			Small/large ratio	Unmyelinated fiber density (no/mm^2)	Tested-fiber study (%)	
	Total fiber	Large fiber	Small fiber			De/re-myelination	Axonal degeneration
1	4557	1778	2779	1.6	19,245	3.0	24.0
2	5728	2594	3134	1.2	13,557	8.0	0.3
3	6085	2845	3240	1.1	17,118	0.5	0.5
4	6902	3530	3372	1.0	13,397	1.1	1.9
5	4807	2766	2041	0.7	21,531	6.2	2.8
Controls (n=10) mean±SD	7087±1413	2717±617	4363±1067	1.7±0.5	30,876±3713	9.0±5.9	1.8±2.0

Control values were obtained from subjects with nonneurologic disease at autopsy.

positive Romberg's sign. Muscle strength was preserved in all patients except for patient 5; this particular patient could not exert full muscle strength in the right lower extremity due to severe pain, and appeared to reveal slight weakness. Autonomic dysfunctions, including constipation, orthostatic hypotension and hypohidrosis were seen in four of the patients. Reduced uptake of ^{123}I-MIBG was evident in two patients (Table 1). Cerebrospinal fluid cell count was normal in all patients, while protein was elevated in two patients (patients 1 and 5). Nerve conduction studies revealed preserved motor and sensory conduction velocities and distal latencies (Table 2). Amplitudes of compound muscle action potential (CMAP) and sensory nerve action potential (SNAP) were greater than the mean±2SD of normal control subjects [11], except for SNAP of the median nerve in patient 1. Sural nerve specimens revealed mild reduction in small-myelinated fibers and unmyelinated fibers in all patients (Fig. 1, Table 3). The density of large myelinated fibers was 2720±627 fibers/mm^2 (100% of mean control values), while that of small myelinated fibers was 2913±535 fibers/mm^2 (66% of mean control values), indicating a predominant reduction in the number of small myelinated fibers. The density of unmyelinated fiber was 16,970±3550 fibers/mm^2 (55% of mean control values). Axonal degeneration was evident in patient 1. There was no evidence of axonal sprouting in any of the patients, suggesting ganglionopathy as a cause of neuropathy. Vasculitis was not observed in any patient.

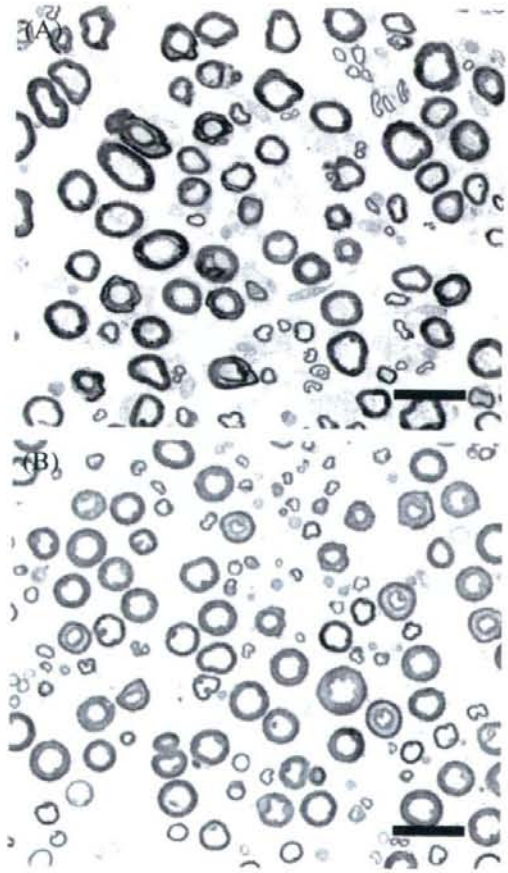

Fig. 1. Sural nerve pathology. (A) Specimen from patient 4. (B) Specimen from a control patient. Specimen from patient 4 revealed predominantly small-fiber loss. No axonal sprouting was seen. Vasculitis was not observed. Scale bar=20 μm.

All patients were treated with 0.4 g/kg intravenous immunoglobulin (IVIg) for 5 days. In all patients, the effect of IVIg treatment was scored by use of the Visual Analogue Scale (VAS) [12]. In addition, we performed quantitative sensory testing (QST) to determine the cold detection threshold (CDT), vibration detection threshold (VDT), and heat-pain (HP) threshold in both the upper and lower extremities using computer aided sensory evaluation version (CASE ; Medical Electronics, Michigan). This evaluation was carried out on one patient (patient 5), before and after treatment. For the CDT, a series of cold stimulation tests, using a range of different temperatures were delivered with a sensor placed on the dorsum of the foot. Patients were asked to respond when the stimulus was felt. The testing algorithms used were the 4, 2, and 1 stepping method for CDT [13], the aim being to determine the smallest temperature differential from the baseline temperature that can be reliably detected. For the VDT, a series of vibration stimulation tests were delivered with a sensor placed on the great toe using the 4, 2, and 1 stepping method. For the HP thresholds, a series of warm stimulation tests were delivered to the dorsum of the foot, using the non-repeating ascending with null stimuli algorithm [14]. HP: 0.5 is the heat-pain detection threshold, HP: 5.0 is a intermediate heat-pain response and the difference between the two (HP: 5.0−0.5). CASE IV normative data were used in accordance with previous studies [13]. Abnormal CDT and VDT were defined as above the 97th percentile (hypoesthesia), and an abnormal HP: 0.5 was defined as below the 3rd percentile (hyperalgesia). QST studies were performed by the same technician in two different patients (patients 4 and 5).

3. Results

All patients responded well to IVIg therapy. Severe pain had been reduced from 7.6±2.9 to 2.2±1.5, according to the Visual Analogue Scale (VAS) (Fig. 2). Several relapses were seen in two patients (patients 1 and 2) over long-term follow up. IVIg treatment was effective at each relapse, but the effect of IVIg became less pronounced in patient 2 after 6 years of treatment (Fig. 2 B). The effect on pain was reported to begin 2 to 14 days after the IVIg infusion started. The clinical improvement lasted for about 2 to 6 months (4.0±2.65 months). The second IVIg therapies were performed in four patients when relapse occurred, with the interval of second IVIg

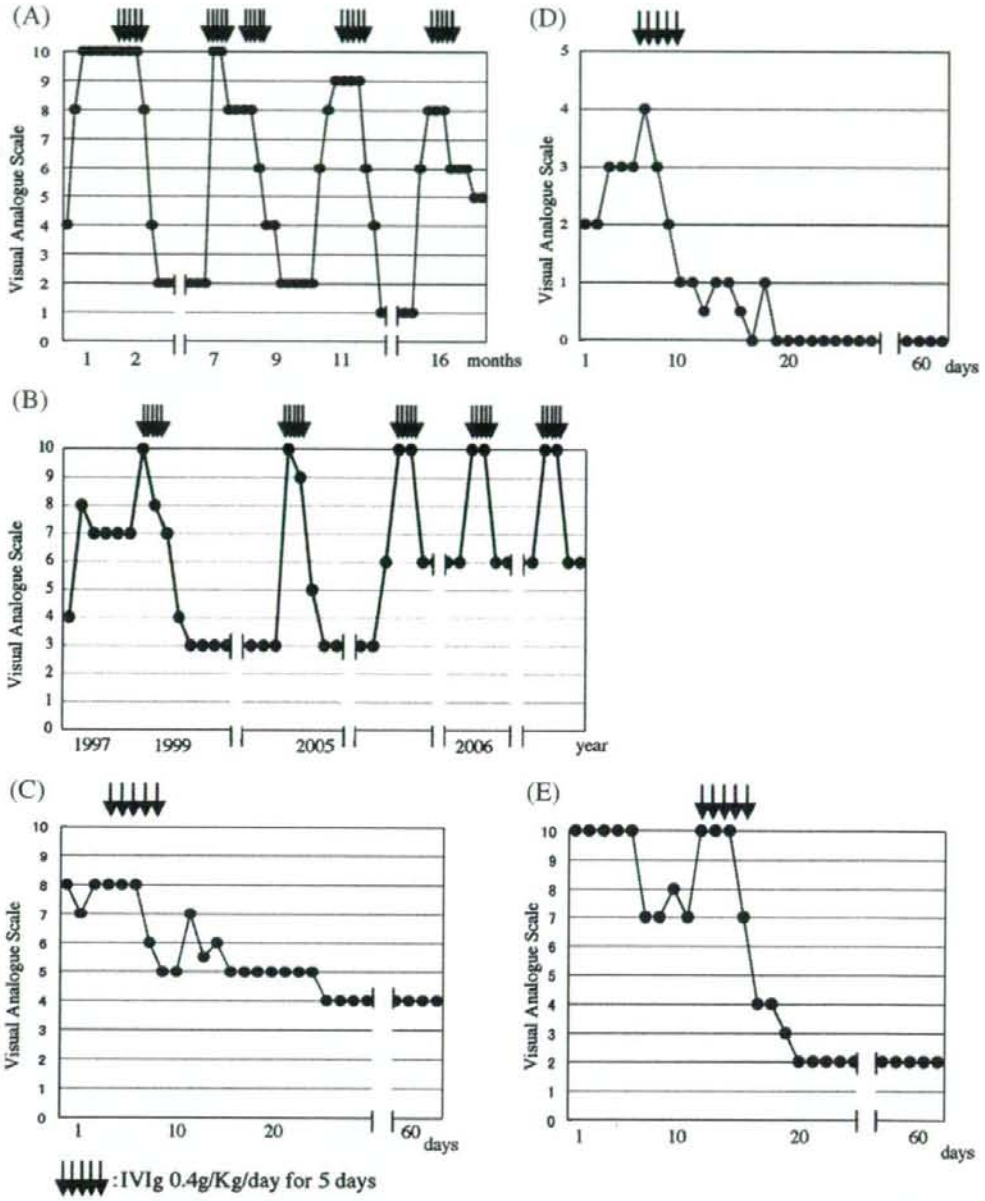

Fig. 2. Clinical course of the Visual Analogue Scale. A 10 cm VAS was anchored by two extremes of pain (left, no pain; right, the worst pain imaginable). Pain ratings were applied subjectively according to the manner described by Kelly [12]. (A) Clinical course of patient 1. (B) Clinical course of patient 2. (C) Clinical course of patient 3. (D) Clinical course of patient 4. (E) Clinical course of patient 5.

treatment ranging from 7 months to 1 year. IVIg therapy reduced pain by 50–100% on the VAS scale; the significant effect of IVIg upon pain relief was clearly evident ($p<0.01$). All patients experienced accompanied superficial sensation, such as numbness, tingling or painful dysaesthesia, but these showed simultaneous improvement. Muscle strength in patient 5 also improved. Following IVIg treatment, patients 1, 3 and 5 were able to walk smoothly and patient 3 no longer required gloves. Direct evidence of sensory improvement was clearly demonstrated by CASE IV analysis in patient 5. VDT 5 was less than +2SD and there was no significant difference before and after treatment. Before treatment, CDT was 28.1 °C (hand) and 10.0 °C (foot), representing abnormal levels greater than +2SD. Following IVIg therapy, CDT improved to 29.8 °C (hand) and 17.3 °C (foot), which was within the normal range of −1.04SD (hand) and +1.88SD (foot). These results clearly demonstrate significant improvement in superficial sensory impairment following IVIg therapy. HP threshold was not different before and after treatment.

4. Discussion

IVIg therapy was effective in alleviating pain symptoms in all 5 patients involved in the present study. Painful symptoms involved

proximal regions of the limbs, face, or trunk in a non-length dependent manner with predominantly superficial sensory involvement. Motor nerve function was well preserved. Pathologically, there was a predominantly small-fiber axon loss with relative preservation of large myelinated axons, without evidence of regenerating fibers. Pathological evaluation of the dorsal root ganglia in patients with major causes of ganglionopathy have been reported for patients with Sjögren's syndrome and paraneoplastic syndrome, via the analysis of tissue obtained by biopsy or autopsy [2,15]. The major symptom in these syndromes is sensory ataxia resulting from the impairment of deep kinaesthetic sensation corresponding to the involvement of large-sized neurons [2,15]. On the other hand, it is uncommon for ganglionopathy to preferentially affect small-diameter neurons [16,17]. However, recent studies have suggested that this type of ganglionopathy may occur in patients with Sjögren's syndrome accompanying painful symptoms [5,17]. Our patients were well concordant with these clinico-pathological features of ganglionopathy with preferential involvement of small-sensory neurons. The concept of ganglionopathy, preferentially involving small neurons, although not yet widely recognized, is rapidly becoming a clinically important field [17].

In our patients, painful symptoms were very severe and significantly interfered with the activity of daily living. Conventional treatments for painful neuropathies, including anticonvulsants, tricyclic antidepressants, SSRI (selective serotonin reuptake inhibitor) or opioids, were not sufficient to ameliorate pain in our patients. Consequently, it was highly evident that other new approaches were needed. Although the mechanisms of pain in painful sensory neuropathy associated with Sjögren's syndrome have yet to be fully clarified, it is considered that immunomodulatory therapy may be effective, based on the hypothesis that painful sensory neuropathy is a continuum of the sensory ataxic form as described above. In the sensory ataxic form, the lesion is located at the level of the sensory ganglion neurons associated with T-cell infiltration [2]. Indeed, IVIg therapy has proved to be effective, to some extent, in the sensory ataxic form [5,18–21]. The putative IVIg effect mechanism includes blockade of the Fc receptor, enhanced antibody catabolism and the suppression of pro-inflammatory cytokines. Therefore, macrophage and B-cell functions would be inactivated and circulating auto-antibodies reduced. IVIg can also exert effect upon superantigens and can modulate T-cell function and antigen recognition [22]. In the ataxic type of Sjögren's syndrome, some of the remaining dorsal root ganglion neurons, which tend to be impaired owing to inflammation, may have regained function because of the IVIg treatment [21]. We speculate that IVIg would elicit the same effect upon small dorsal root ganglion neurons in painful Sjögren's syndrome-neuropathy.

Pro-inflammatory cytokines such as TNF-alpha, IL-1 beta, and IL-6 contribute to the development of inflammatory and neuropathic pain, and hyperalgesia [23,24]. Indeed, in patients with painful neuropathy, or complex regional pain syndrome, TNF-alpha has been reported to correlate with the presence of mechanical hyperalgesia [25,26]. However, participation of such cytokines in painful sensory neuropathy associated with Sjögren's syndrome remains largely unknown. In one of our patients, some serum cytokines, e.g., TNF-α, IL-8 were shown to be reduced by IVIg (patient 3, data not shown). A question of IVIg treatment is cost. It is certain that IVIg is very expensive, but IVIg bring rapid and sufficient improvement. Therefore, IVIg treatment is considered to be useful for patients who have severe pain or have insufficient improvement by conventional treatment. Therefore, IVIg might be an effective treatment for pain in Sjögren's syndrome-associated neuropathy. Further studies should be done in a controlled, blind study.

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Phase 2 Trial of Leuprorelin in Patients with Spinal and Bulbar Muscular Atrophy

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Objective: Spinal and bulbar muscular atrophy (SBMA) is a hereditary motor neuron disease caused by the expansion of a polyglutamine tract in the androgen receptor (AR). Animal studies have shown that the pathogenesis of SBMA is dependent on serum testosterone level. This study is aimed at evaluating the efficacy and safety of androgen deprivation by leuprorelin acetate in patients with SBMA.

Methods: Fifty SBMA patients underwent subcutaneous injections of leuprorelin acetate or placebo in a randomized, placebo-controlled trial for 48 weeks, followed by an open-label trial for an additional 96 weeks, in which 19 patients of the leuprorelin group and 15 of the placebo group received leuprorelin acetate. The patients who did not participate in the open-label trial were also followed up for the 96-week period (UMIN000000474).

Results: Leuprorelin acetate significantly extended the duration of cricopharyngeal opening in videofluorography and decreased mutant AR accumulation in scrotal skin biopsy. The patients treated with leuprorelin acetate for 144 weeks exhibited significantly greater functional scores and better swallowing parameters than those who received placebo. Autopsy of one patient who received leuprorelin acetate for 118 weeks suggested that androgen deprivation inhibits the nuclear accumulation or stabilization, or both, of mutant AR in the motor neurons of the spinal cord and brainstem.

Interpretation: These observations suggest that administration of leuprorelin acetate suppresses the deterioration of neuromuscular impairment in SBMA by inhibiting the toxic accumulation of mutant AR. The results of this phase 2 trial support the start of large-scale clinical trials of androgen deprivation for SBMA.

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Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is the first of the neurodegenerative diseases for which the molecular basis was discovered to be the expansion of a trinucleotide CAG repeat in the gene of the causative protein. SBMA is an adult-onset, motor neuron disease characterized by muscle atrophy, weakness, contraction fasciculations, and bulbar involvement.[1–4] Its prevalence has been estimated to be 1 to 2 per 100,000, although a considerable number of patients may be misdiagnosed with other neuromuscular diseases such as amyotrophic lateral sclerosis (ALS).[5,6] The progression of SBMA is usually slow, but life-threatening respiratory tract infections often occur in the advanced stage of the disease, resulting in death.[7] Laboratory tests show increased serum levels of creatine kinase and liver enzymes in most cases. The expanded CAG triplet repeat sequence, which encodes a polyglutamine tract, is found in the androgen receptor gene *(AR)*.[8] The CAG repeat numbers range from 38 to 62 in SBMA patients, whereas healthy individuals have 9 to 36 CAGs.[5,8–10] The number of CAGs is correlated with disease severity and inversely correlated with the age of onset, as observed in other polyglutamine-related neurodegenerative dis-

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eases, including Huntington's disease and several forms of spinocerebellar ataxia.[11,12] In polyglutamine diseases, nuclear localization of the respective mutant protein is considered important for inducing neuronal cell dysfunction and degeneration.[13–15] The extent of diffuse nuclear accumulation of mutant AR in spinal motor neurons is closely related to the CAG repeat length in autopsied SBMA cases.[16] In addition, nuclear accumulation of mutant AR in scrotal skin correlates with both disease severity and CAG repeat length, suggesting that the number of scrotal skin epithelial cells positive for 1C2, an anti-polyglutamine antibody, is a potent pathogenic marker of SBMA and can serve as a useful biomarker in therapeutic trials.[17]

A characteristic clinical feature of SBMA is that full disease manifestations occur in male but not in female individuals even when they are homozygous for the mutation.[18,19] The sex dependency of disease manifestation in SBMA may arise from a testosterone-dependent nuclear accumulation of mutant AR.[20–23] In mouse models of SBMA, surgical castration delays disease onset and progression, as well as reverses neuromuscular phenotypes.[20,23] Leuprorelin acetate, a luteinizing hormone–releasing hormone agonist that reduces testosterone release from the testis and inhibits nuclear accumulation of mutant AR, ameliorates motor dysfunction in male transgenic mice carrying full-length mutant human *AR* with an expanded polyglutamine tract.[21]

Although data from animal studies indicated that androgen deprivation via leuprorelin acetate is a promising therapeutic agent for SBMA,[20,21] clinical experience using this drug in SBMA patients is limited.[24] The safety and efficacy of leuprorelin acetate were demonstrated for treating prostate cancer, endometriosis, uterine fibroids, and central precocious puberty in children.[25] To determine whether androgen deprivation therapy prevents the progression of SBMA in humans, we conducted a two-arm, randomized, placebo-controlled, phase 2 clinical trial of leuprorelin acetate in patients with SBMA for 48 weeks, followed by an open-label trial for an additional 96 weeks.

Patients and Methods

Patients

Inclusion criteria of this trial included: (1) genetically confirmed SBMA male Japanese patients with more than one of the following symptoms: muscle weakness, muscle atrophy, bulbar palsy, or hand tremor; (2) patients whose *AR* CAG repeat length was more than 38; (3) patients who were 30 to 70 years old at the time of informed consent; (4) patients who had no desire to father a child; and (5) patients who gave written informed consent. Patients were excluded if they met any of the following criteria: (1) medical history of allergy to leuprorelin acetate; (2) had taken testosterone within 8 weeks before the informed consent; (3) had severe complications; or (4) were not eligible for other reasons (eg, previous use of luteinizing hormone–releasing hormone agonists or medical history of allergy to barium sulfate).

Study Design

We conducted a 48-week, prospective, randomized, placebo-controlled, single-site trial and a 96-week open-label follow-up trial at Nagoya University Hospital (Fig 1). Fifty patients were included between September 2003 and March 2004. The last patient terminated the randomized controlled trial (RCT) in February 2005. The protocol for the trial was filed with the open clinical trial registry (www.umin.ac.jp/ctr/index.htm) under the Identifier Number UMIN000000474. In the 48-week RCT, patients were randomized in a 1:1 ratio of leuprorelin acetate or identically appearing placebo using the minimization method by an independent investigator. Dynamic allocation was performed based on patient age and severity to reduce bias.[26] Patients were blinded throughout the RCT, and at week 48, they decided whether to participate in the follow-up trial without knowing to which drug group they had been allocated. As a result, 19 patients in the leuprorelin group and 15 in the placebo group entered the open-label follow-up trial between August 2004 and March 2005. The remaining 15 patients who declined to participate in the open-label trial were followed up for these 96 weeks; 1 patient who discontinued early in the 48-week RCT was not followed up. The last patient terminated the follow-up trial in February 2007.

All the examinations and treatments were performed at the Nagoya University Hospital throughout the trials. The patients were hospitalized for 7 days at weeks 0 and 48, and were evaluated every 4 weeks in the 48-week RCT. During the 96-week follow-up trial, they were examined every 12 weeks. Blinding was ensured by the use of identical opaque injection syringes. Clinical scores and muscle strength were assessed by blinded neurologists throughout the RCT period.

Treatment

Leuprorelin acetate was subcutaneously injected at a dose of 3.75mg every 4 weeks in the 48-week RCT, and 11.25mg was administrated every 12 weeks in the 96-week follow-up trial. Leuprorelin acetate suppresses testosterone release by downregulating luteinizing hormone–releasing hormone receptors in the pituitary. We did not conduct a dose–response study in this trial, because previous studies suggested that leuprorelin-mediated androgen deprivation is incomplete at dosages less than 3.75mg/4 weeks in men.[27,28]

Outcome Measures

The primary end point of this trial was motor function measured by the widely used and validated Revised ALS Functional Rating Scale (ALSFRS-R; Japanese edition). Although there are no validated scales for SBMA, all the items in the ALSFRS-R are applicable to this disease.[17,29,30] Secondary outcome measures included cricopharyngeal opening duration visualized by videofluorography (VF), the frequency of 1C2-positive cells in scrotal skin biopsies, lung function values [forced expiratory volume in 1 second/forced vital capacity (FEV_1/FVC) and vital capacity as the percentage of pre-

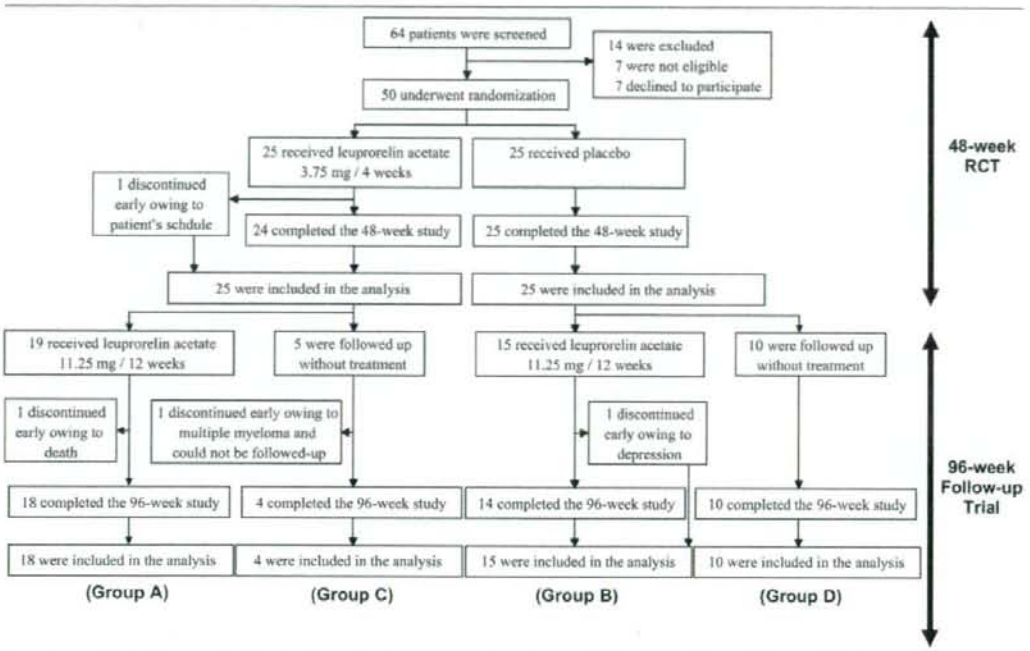

Fig 1. Patient selection flow diagram. RCT = randomized controlled trial.

dicted (%VC)], and serum levels of the following enzymes: L-aspartate aminotransferase, L-alanine aminotransferase, and creatine kinase. As other outcome measures, we analyzed the sum of the following three ALSFRS-R subscores: speech, salivation, and swallowing. We also measured muscle strength using maximum voluntary isometric contraction and conducted a nerve conduction study. The Beck depression inventory and standard laboratory parameters were checked for evaluating safety. Scrotal skin biopsies were performed at weeks 0 and 48 of the 48-week RCT. The ALSFRS-R, lung function test, maximum voluntary isometric contraction, and Beck depression inventory were measured every 24 weeks throughout the trials, whereas VF was examined at weeks 0 and 48 in the 48-week RCT, and at the end of the 96-week follow-up trial. The nerve conduction study was conducted at weeks 0 and 48 in the 48-week RCT. All laboratory tests were performed at weeks 0, 4, 8, 12, 24, 36, and 48 in the 48-week RCT, and every 12 weeks in the 96-week follow-up trial. Outcome for the efficacy analysis was assessed at the final visit at the end of 48-week RCT and at the end of the 96-week follow-up trial.

In VF examinations, patients were instructed to swallow 3ml of 40% wt/vol barium sulfate twice in a standing position. VF data were recorded on Mini DV videotape (Sony, Tokyo, Japan) at 30 frames/sec. For scrotal skin biopsies at 0 and 48 weeks, three specimens were taken from each patient at each time by punch biopsy using a 3mm diameter Dermapunch (Nipro, Tokyo, Japan) under local anesthesia (lidocaine acetate, 10ml) and processed for immunohistochemical analysis using an anti-polyglutamine (1C2) antibody, as described later. All 50 patients underwent biopsies at week 0, but 2 patients' specimens did not attach to the slides and were not included in the analyses. All patients who underwent biopsy sterilized the wound for several days themselves and underwent antibiotic therapy after the procedure, as needed. Serum creatine kinase levels were determined in blood samples obtained on the second day of admission by ultraviolet measurement using hexokinase and glucose-6-phosphate.[31] Serum testosterone levels were measured by radioimmunoassay using the DPC total testosterone kit (Diagnostic Products Corporation, Los Angeles, CA).

Quantitative Measurement in Videofluorography

To identify the appropriate parameters for the outcome measure in this trial, we performed a preliminary analysis of quantitative swallowing evaluation using VF in 18 additional SBMA patients (see Supplemental Tables 1 and 2). In this preliminary study, we assessed the reliability and validity of pharyngeal residue and those of the various temporal measures: pharyngeal delay time, cricopharyngeal opening duration, and total duration of maximal laryngeal elevation. As a result, we found that cricopharyngeal opening duration was the most reliable measurement of swallowing, and that this duration correlated well with functional scores such as the Norris Scores and the ALSFRS-R. None of the other parameters exhibited both high reliability and correlation with functional scores. Therefore, we adopted cricopharyngeal

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

Immunohistochemical Detection of Mutant Androgen Receptor

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

Autopsy Study

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

Genetic Analysis

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

Statistical Analyses

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

Ethics

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

Results

Demographics

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

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

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

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

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

Forty-eight-Week Randomized Controlled Trial

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

Ninety-six-Week Follow-up Trial

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

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

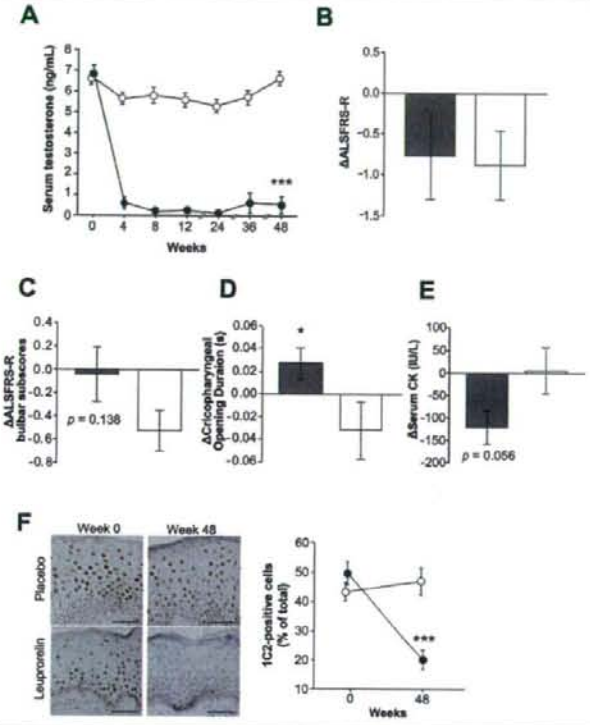

Fig 2. Efficacy results of the 48-week randomized controlled trial (RCT). (A) Treatment with leuprorelin acetate (black circles) *rapidly depleted serum testosterone levels.* White circles *represent placebo group. (B) There was no significant difference in the change in Revised Amyotrophic Lateral Sclerosis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

Safety and Tolerability

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

Autopsy Study

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

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

	Leuprorelin in 48-week RCT (n = 22)			Placebo in 48-week RCT (n = 25)		
Treatment in 96-week Follow up	**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.

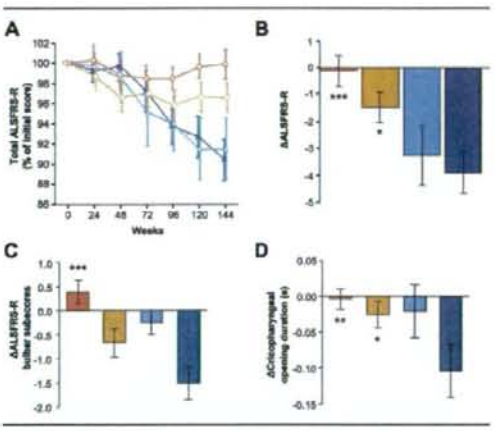

*Fig 3. Efficacy results of the 96-week follow-up trial. (A, B) Changes in the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) scores showed treatment duration–dependent improvements in the leuprorelin-treated groups. (C, D) The ALSFRS-R bulbar subscores (C) and videofluorography (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).*

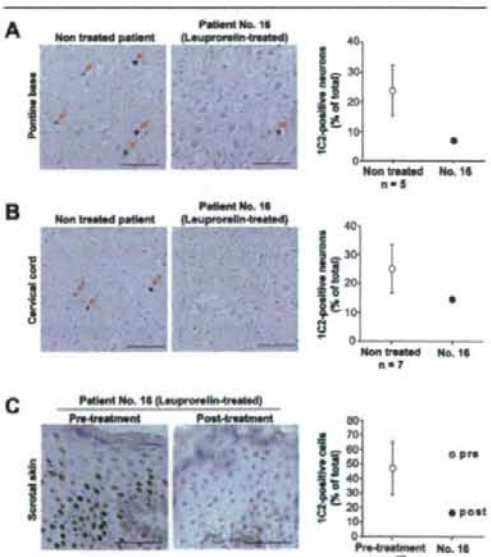

Fig 4. Effects of leuprorelin acetate on nuclear accumulation of mutant androgen receptor (AR). (A, B) Accumulation of mutant AR in neurons was remarkable both in the pontine base and in the spinal anterior horn of all the control, non-treated autopsied cases, but the number of 1C2-positive neurons was 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*	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%)

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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ABSTRACT

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

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

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

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

GLOSSARY

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

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

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

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

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

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

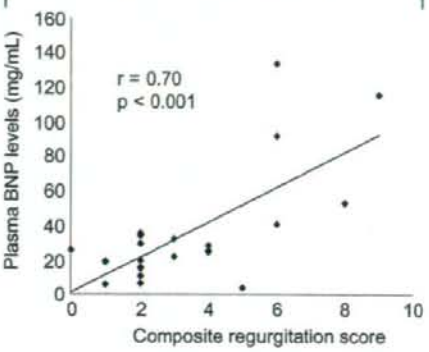
* The frequency of any grade 3 to 4 regurgitation was statistically significant between the ergot group and the non-ergot group ($p < 0.0001$) as well the ergot group and the control group ($p < 0.001$).

† 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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