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Table 1 Clinical features and MUNE of SBMA patients and normal controls

		SBMA		normal		p
		(Mean ± SD)	n	(Mean ± SD)	n	
Age at examination	(years)	53.2 ± 10.9	52	48.8 ± 11.0	13	NS
Disease duration	(years)	10.5 ± 7.7	52			
CAG repeat size in *AR* gene	(number)	48.2 ± 3.2	43*			
Limb Norris score (normal score = 63)		53.7 ± 7.4	52	63.0 ± 0.0	13	<0.001
Norris Bulbar score (normal score = 39)		33.0 ± 4.2	52	39.0 ± 0.0	13	<0.001
ALSFRS-R (normal score = 48)		40.6 ± 4.1	52	48.0 ± 0.0	13	<0.001
Right grip power	(kg)	20.1 ± 5.9	52	45.2 ± 5.2	13	<0.001
Left grip power	(kg)	19.1 ± 6.0	52	42.4 ± 5.5	13	<0.001
MUNE (amplitude)		71.8 ± 40.0	52	313.9 ± 129.9	13	<0.001
MUNE (area of negative portion**)		87.8 ± 63.5	52	341.7 ± 157.2	13	<0.001
maximum CMAP	(mV)	7.3 ± 2.1	52	10.5 ± 2.0	13	<0.001
averaged SMUP	(μV)	144.1 ± 104.4	52	38.0 ± 15.9	13	0.001

* In the remaining 9 patients, the diagnosis of SBMA was genetically confirmed using agarose gel electrophoresis, but CAG repeat size was not calculated.

** The area of the first negative phase was calculated.

AR, androgen receptor; ALSFRS-R, ALS functional rating scale-revised; MUNE, motor unit number estimation; CMAP, compound muscle action potentials; SMUP, single motor unit potentials; NS, not significant

Table 2 Correlation coeficients between MUNE and backgrouds in SBMA patients

	MUNE caluculated by the amplitude (p value)	MUNE caluculated by the area (p value)
vs. age at examination	0.00 (NS)	0.18 (NS)
vs. disease duration	-0.44 (0.001)	-0.26 (NS)
vs. CAG repeat size	-0.18 (NS)	-0.32 (0.034)
vs. Limb Norris score	0.10 (NS)	-0.12 (NS)
vs. Norris Bulbar score	0.09 (NS)	-0.06 (NS)
vs. ALSFRS-R	0.11 (NS)	-0.09 (NS)
vs. ipsilateral grip power (right)	0.45 (0.001)	0.18 (NS)
vs. contralateral grip power (left)	0.23 (NS)	0.03 (NS)

MUNE was recorded from the right hand of all the patients. ALSFRS-R, ALS functional rating scale-revised; MUNE, motor unit number estimation; NS, not significant

Table 3 Natural history of MUNE and motor function

A	initial exam (Mean ± SD)	one-year follow-up (Mean ± SD)	n	*p*
MUNE				
amplitude	77.2 ± 31.3	54.5 ± 26.0	11	0.002
area of negative portion	92.3 ± 56.9	51.6 ± 26.9	11	0.003
maximum CMAP (mV)	6.6 ± 2.5	5.7 ± 2.5	11	NS
averaged SMUP (μV)	108.6 ± 56.2	125.5 ± 33.5	11	NS
Motor function				
Limb Norris Score	56.3 ± 6.2	54.3 ± 8.4	11	NS
Norris Bulbar Score	32.9 ± 5.9	32.7 ± 5.7	11	NS
ALSFRS-R	41.2 ± 4.1	40.7 ± 5.3	11	NS
Right grip power (kg)	22.1 ± 4.7	21.3 ± 4.7	11	NS

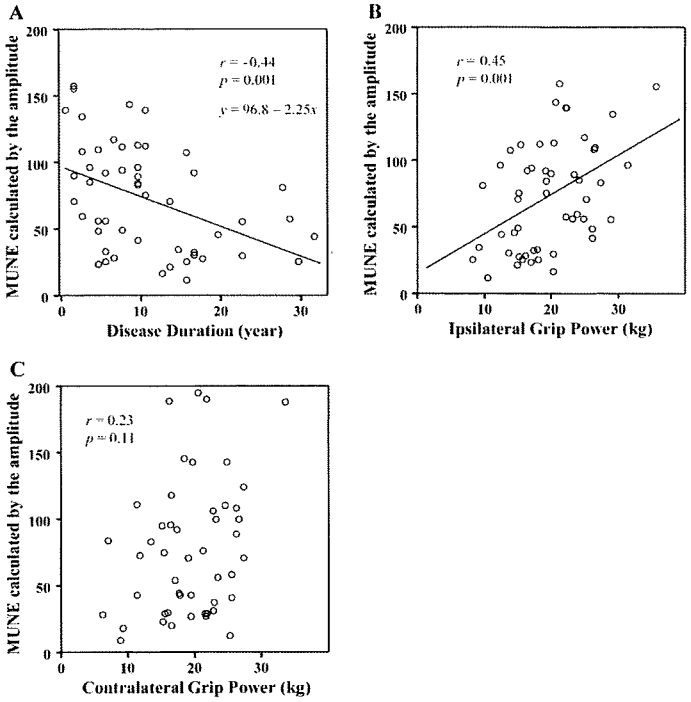
B	followed-up group (Mean ± SD)	n	non followed-up group (Mean ± SD)	n	*p*
Age at examination (years)	53.2 ± 14.4	11	53.2 ± 9.9	41	NS
Disease duration (years)	8.0 ± 4.1	11	12.0 ± 8.3	41	NS
CAG repeat size in *AR* gene (number)	48.9 ± 4.6	10	48.0 ± 2.7	33	NS
Limb Norris score (normal score = 63)	56.3 ± 6.2	11	53.0 ± 7.6	41	NS
Norris Bulbar score (normal score = 39)	32.9 ± 5.9	11	33.0 ± 3.7	41	NS
ALSFRS-R (normal score = 48)	41.2 ± 4.1	11	40.5 ± 4.1	41	NS
Right grip power (kg)	22.1 ± 4.7	11	19.6 ± 6.2	41	NS

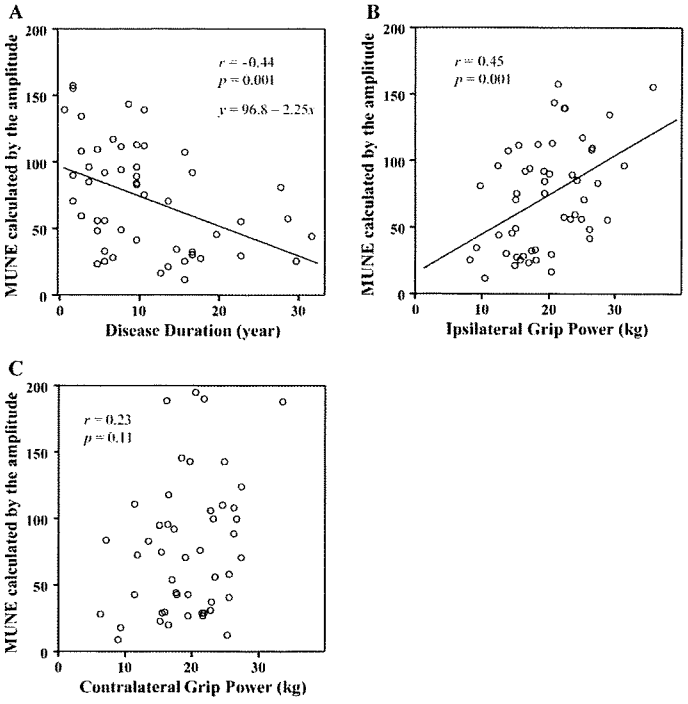
MUNE, motor unit number estimation; CMAP, Compound muscle action potentials; SMUP, single motor unit potentials; ALSFRS-R, ALS functional rating scale-revised; AR, androgen receptor; NS, not significant

Supplemental table 1 Frequency of patients with abnormal MUNE values

	MUNE		maximum CMAP
	caluculated by the amplitude	caluculated by the area	
Less than mean - 1SD	100% (52/52)	84.6% (44/52)	65.4% (34/52)
Less than mean - 2SD	38.5% (20/52)	15.4% (8/52)	38.5% (20/52)

MUNE, motor unit number estimation; CMAP, Compound muscle action potentials


A
MUNE calculated by the amplitude
200
150
100
50
0
0
10
20
30
Disease Duration (year)
r = -0.44
p = 0.001
y = 96.8 – 2.25x
B
MUNE calculated by the amplitude
Ipsilateral Grip Power (kg)
r = 0.45
p = 0.001
C
MUNE calculated by the amplitude
Contralateral Grip Power (kg)
r = 0.23
p = 0.11


A
MUNE calculated by the amplitude
200
150
100
50
0
0
10
20
30
Disease Duration (year)
r = -0.44
p = 0.001
y = 96.8 – 2.25x
B
MUNE calculated by the amplitude
Ipsilateral Grip Power (kg)
r = 0.45
p = 0.001
C
MUNE calculated by the amplitude
Contralateral Grip Power (kg)
r = 0.23
p = 0.11

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-

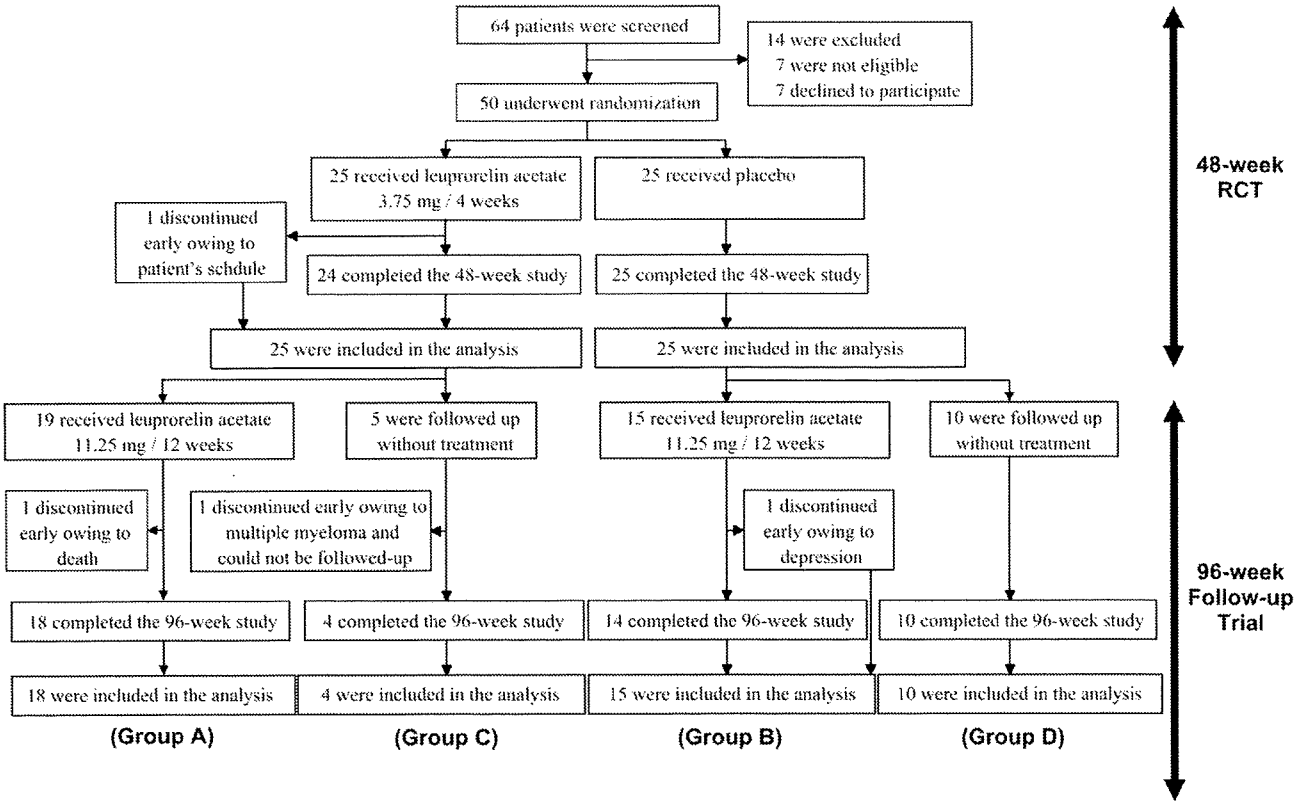

Fig 1. Patient selection flow diagram. RCT = randomized controlled trial.

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).

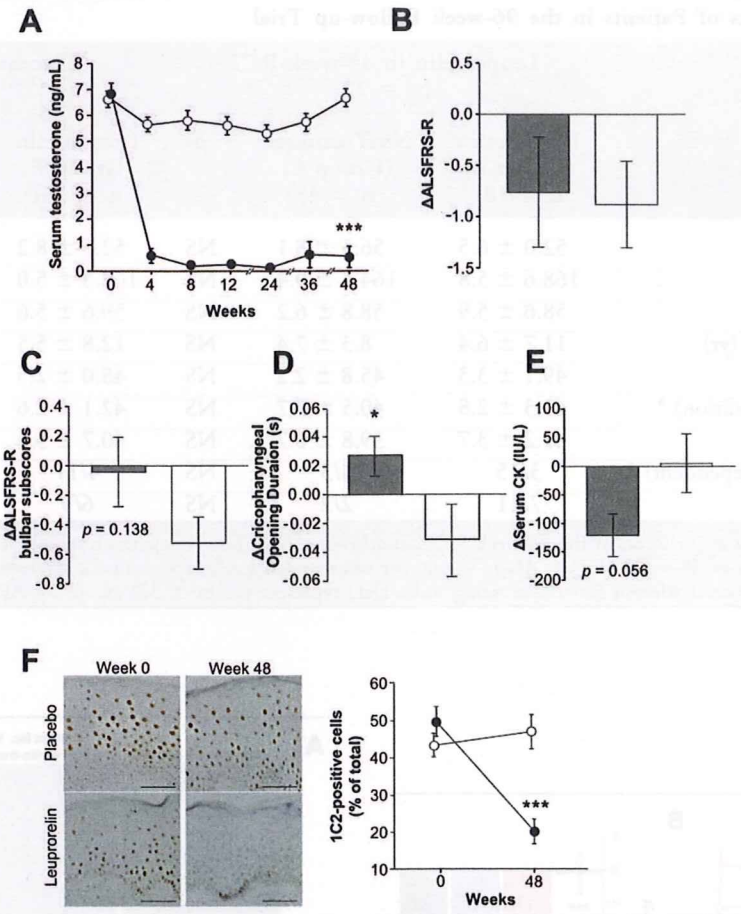

Fig 2. Efficacy results of the 48-week randomized controlled trial (RCT). (A) Treatment with leuprorelin acetate (black circles) *rapidly depleted serum testosterone levels.* White circles *represent placebo group. (B) There was no significant difference in the change in Revised Amyotrophic Lateral Sclerosis 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**

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.

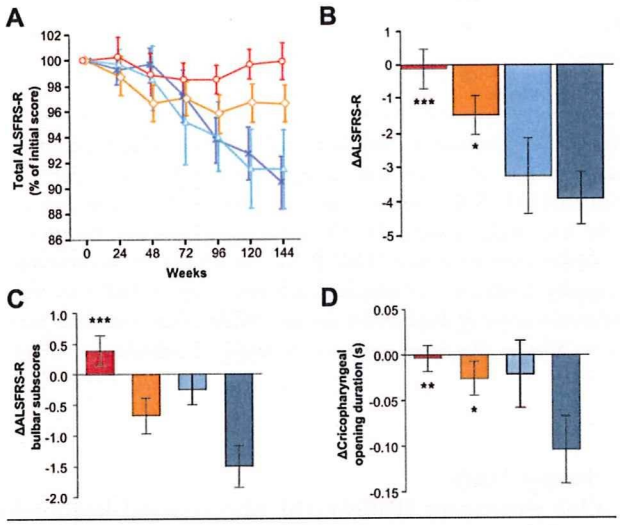

*Fig 3. Efficacy results of the 96-week follow-up trial. (A, B) Changes in the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) scores showed treatment duration–dependent improvements in the leuprorelin-treated groups. (C, D) The ALSFRS-R bulbar subscores (C) and videofluorography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

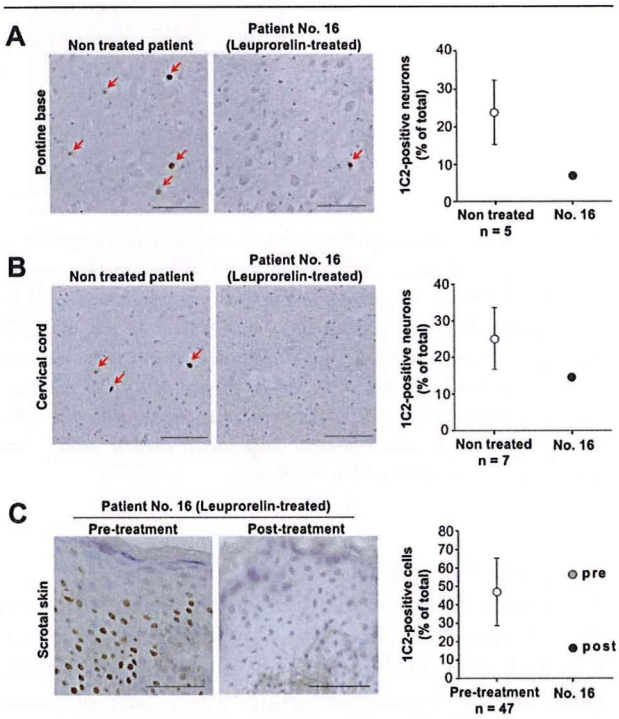

Fig 4. Effects of leuprorelin acetate on nuclear accumulation of mutant androgen receptor (AR). (A, B) Accumulation of mutant AR in neurons was remarkable both in the pontine base and in the spinal anterior horn of all the control, non-treated autopsied cases, but the number of 1C2-positive neurons was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