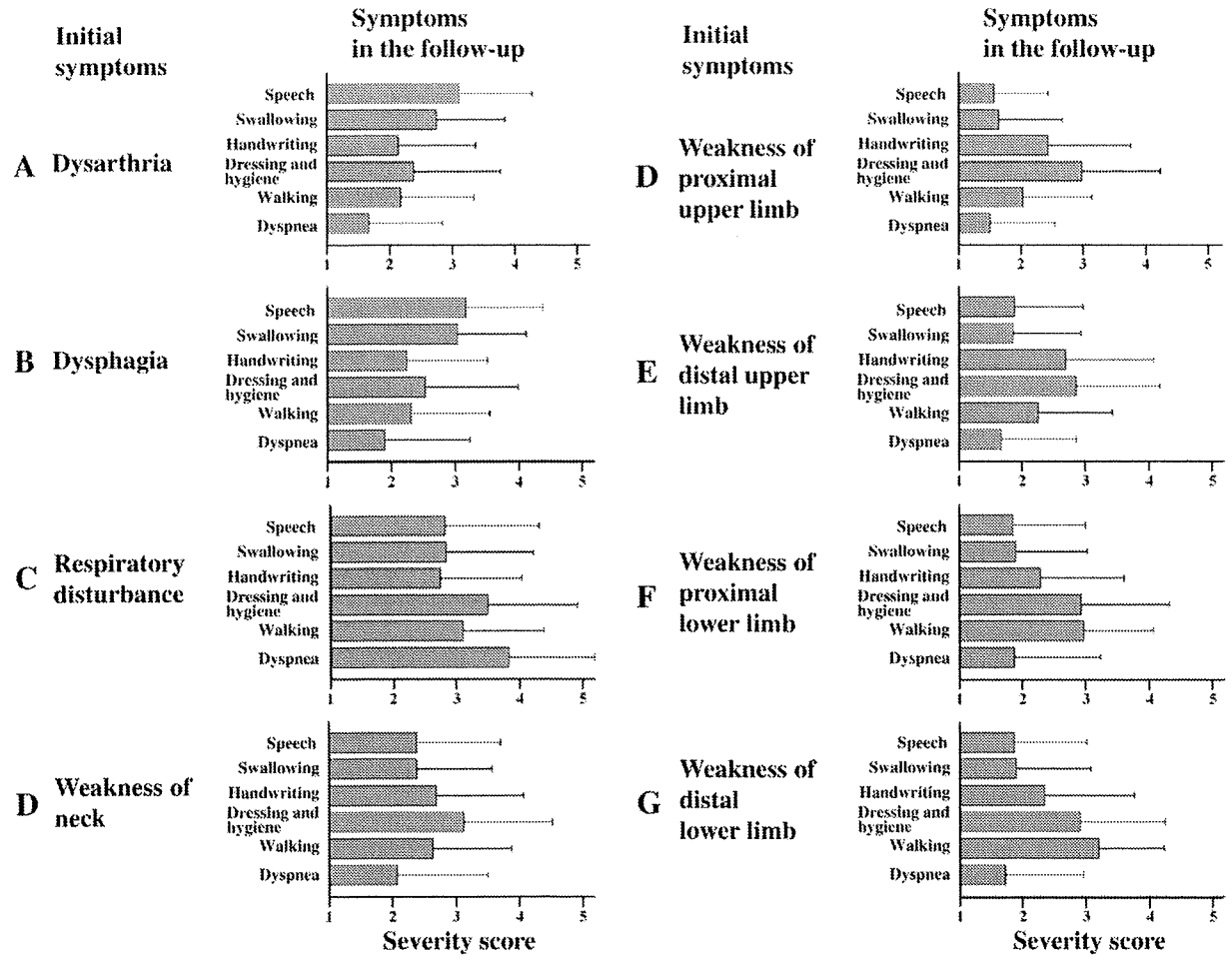

Fig. 4. Relationship between initial symptoms and symptoms at the follow-up stage. Severity scores of Speech, Swallowing, Handwriting, Dressing and Hygiene, Walking and Dyspnea are shown as subscales of ALSFRS-R. The score of "5" represents the most severe state, and "1" represents the absence of the symptom. Initial symptoms remain the most prominent or related symptoms even in the follow-up stage for 1.7±2.2 years from onset, suggesting that initial symptoms significantly determine the prominent features of symptoms throughout the disease course. $n=3428$.

(Fig. 3A,B). On the other hand, weakness in the upper or lower limbs as an initial symptom was seen more frequently in patients with a younger age at onset, and these frequencies gradually decreased with increasing age at onset. As for the respiratory disturbance and dropping head due to weakness of the neck muscles, the frequencies increased gradually with increasing age at onset. When we divided the patients between those with an onset age of older than 65 years and those younger than 65 years and analyzed the data with the chi-square test, the differences in frequencies of dysarthria, dysphagia, respiratory disturbance, weakness of upper extremities and weakness of lower extremities as initial symptoms were also significant between those groups ($p<0.001$, $p<0.001$, $p<0.001$, $p=0.001$, $p=0.019$, respectively). The difference in the frequency of neck weakness was not significant ($p=0.07$), although the tendency was apparent, and may be due to the small number of patients with neck weakness as an initial symptom. These observations suggest that age at onset is a determining factor of the features of the initial symptoms. Correlations between age at onset and the frequency of initial symptoms were similarly observed in the male and female patient groups (Supple. Fig. 1).

3.5. Initial symptoms determine major clinical features in follow-up stage

We examined the relationship between the initial symptoms and the symptoms assessed by 6 items of ALSFRS-R at examination at 1.7±2.2 years after the onset (Fig. 4). At the follow-up stage, the patients who showed a bulbar symptom as an initial symptom showed speech or swallowing disturbance as a major symptom in the follow-up stage. Patients that showed respiratory disturbance as an initial symptom also showed dyspnea as the most prominent disturbance; patients with weakness of distal upper limb muscles showed the most prominent disturbance in handwriting and dressing; patients with weakness of proximal upper limbs showed prominent disturbance in dressing and hygiene; and patients with weakness of lower limbs, either proximal or distal, all showed a prominent disturbance in walking. These observations strongly suggested that the initial symptoms remained the most prominent or related symptoms even in the follow-up stage, and support the view that the initial symptoms determine the clinical features of the individual patient even in the follow-up stage. A similar tendency was observed in the male and female patient groups (Supple. Fig. 2).

4. Discussion

The results of the present study demonstrate the characteristic clinical profiles of Japanese sporadic ALS patients. A very high rate of Japanese ALS patients (29.3%) were under TPPV compared to patients in North America or Europe [10,11,17,18] which are 2.1–5.4%, respectively. The frequency of patients showing rare symptoms such as ophthalmoplegia increased with disease progression, particularly under long-standing TPPV.

A striking observation in the present study is that the age at onset greatly influences the wide-ranging clinical features, including the initial symptoms, progression to the endstage assessed by introduction of TPPV, and the frequency of rare symptom in the long-standing course. A higher incidence of bulbar involvement in patients with an older age at onset has been reported in some previous studies [19–23]. We extended these observations in that almost all of the initial symptoms, such as dysphagia, dysarthria, upper or lower limb weakness, respiratory failure and head dropping are strongly influenced by the age at onset. This observation was also confirmed in the subpopulation of male and female patients. In addition, since the initial symptoms also determine the prominent clinical phenotypes in the follow-up stage as demonstrated in this study, age at onset may influence not only the initial symptoms, but also the entire clinical phenotypes of sporadic ALS. The underlying mechanism for the onset age influence on the initial manifestation of the symptoms is unknown. Furthermore, we do not know the mechanism by which patients with a younger age at onset tend to show a higher frequency of rare symptoms. Further study is needed to resolve these issues, although one may speculate that subpopulations of the motor neurons may be differentially vulnerable to the aging process. In several sporadic neurodegenerative diseases, age at onset has been suggested to be an influencing factor for the spatial development of neural involvement, and, thus, for the features of clinical manifestations [24]. In Parkinson's disease, for instance, patients with an older age at onset have been suggested to have a tendency to show a higher cognitive dysfunction and autonomic dysfunction [25–27], whereas, those with a younger age at onset have an increased tendency toward dystonia and a diurnal fluctuation of symptoms [28,29]. Taking these observations together with our findings on ALS, age at onset may be a more important factor modifying clinical manifestations in sporadic neurodegenerative diseases than previously thought.

Age at onset also influenced the interval from the onset to the time of introduction of TPPV. Reserved respiratory function is known to decrease with advancing age [19]. Therefore, the short interval between the onset and the introduction of TPPV may be explained by the smaller reserved respiratory capacity in elderly patients. Indeed, serial examinations of the respiratory function in elderly patients start at a lower vital capacity and reach a critical point more quickly than younger patients [19,30]. It is congruent with the fact shown in the previous reports [1,3,5,6,22], that younger ALS patients survive longer than older patients.

Therefore, in taking into account the age at onset, initial symptoms, occurrence of rare symptoms and progression, the age at onset greatly affects the clinical profiles of sporadic ALS patients. In addition, the onset age-related initial symptoms are important to estimate the patient's prognosis as well as the design of clinical trials [31].

A high proportion of ALS patients in Japan are under TPPV compared to patients in other countries, possibly for social, cultural and economic reasons [13,17,18]. The presence of a subgroup of patients extending involvement to other systems beyond motor neurons, such as oculomotor, autonomic, sensory and higher functional systems, has been described in Japanese ALS patients under long-term TPPV treatment [32–36]. Pathologically, these patients show an extensive involvement of the tegmentum of the brainstem, substantia nigra, Clarke's dorsal nuclei and spinocerebellar tract, and frequent involvement of the thalamus and globus pallidus. Our present observations have confirmed these reports on sporadic Japanese ALS patients, particularly those with long-standing TPPV, and demonstrated that these subpopulations with a rare extension of involvements include almost 30% of the patients with 9 years or more under TPPV, particularly those assessed for oculomotor system involvement. However, further studies are needed to determine whether all the patients would eventually show an extended involvement beyond the motor system or whether these patients with an extended form are restricted to a given subpopulation. This is an important issue to determine the natural history of sporadic ALS. Since European and American ALS patients are not generally maintained on TPPV treatment for a longer period as Japanese patients, extended involvement is very rarely observed in Europe or North America.

In summary, we have presented the clinical profiles of sporadic Japanese ALS patients based on a large-scale sample. As demonstrated, age at onset may be a remarkable factor influencing wide-ranging clinical profiles including the progression and prognosis. We should take account of this observation in cohort studies or clinical trials.

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Appendix A

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Appendix B. Supplementary data

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

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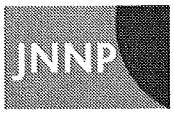

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The profile of motor unit number estimation (MUNE) in spinal and bulbar muscular atrophy

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Key words: motor neuron disease, spinal and bulbar muscular atrophy (SBMA), motor unit number estimation (MUNE), natural history, biomarker

Word Count: 2299

Abstract

Objective: Spinal and bulbar muscular atrophy (SBMA) is a lower motor neuron disease caused by the expansion of a trinucleotide CAG repeat in the androgen receptor (*AR*) gene. The fundamental histopathological finding of this disease is an extensive loss of lower motor neurons in the spinal cord and brain stem. It is, however, difficult to clinically evaluate the degree of motor neuron degeneration, which stresses the need for biomarkers to detect the remaining neuronal function.

Methods: We performed motor unit number estimation (MUNE) in 52 SBMA patients to investigate whether this method could be a potential biomarker of SBMA. We also re-evaluated MUNE one year later in a subgroup of the patients.

Results: The number of functioning motor units was remarkably reduced in SBMA patients compared to controls, and was correlated with both ipsilateral grip power and disease duration. A longitudinal analysis demonstrated a further reduction of motor units within one year.

Conclusions: Our results suggest that MUNE is an electrophysiological parameter that reflects the severity and progression of motor neuron degeneration in patients with SBMA.

Introduction

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is a hereditary lower motor neuron disease affecting adult males.[1-3] The cause of SBMA has been identified as the expansion of a trinucleotide CAG repeat in the androgen receptor (*AR*) gene.[4] The number of CAGs in the *AR* gene is 9 - 36 in normal subjects and 38 - 62 in SBMA patients.[5, 6] In SBMA patients, there is also an inverse correlation between the number of CAGs and the age at onset.[7, 8] The main symptoms of SBMA are weakness and atrophy of the bulbar, facial and limb muscles.[9] In addition to motor symptoms, sensory impairment, such as vibratory sensory disorder, is often observed.[10, 11] Patients occasionally demonstrate signs of androgen insensitivity such as gynecomastia and testicular atrophy. The onset of muscle weakness is usually between 30 and 60 years, but is often preceded by nonspecific symptoms such as postural tremor and muscle cramps.[8, 9] The progression of SBMA is usually slow, with a considerable number of patients needing assistance to walk by the time they are 50 - 60 years old. A susceptibility to aspiration pneumonia increases as bulbar paralysis develops, and the most common cause of death in SBMA patients is pneumonia.[8, 12] Many patients have hypertension, hyperlipidemia, liver dysfunction, and glucose intolerance. In addition, the serum creatine kinase level is elevated in the majority of patients. Electromyograms of patients with SBMA show neurogenic abnormalities such as high amplitude potentials, reduced interference, and polyphasic

potentials. Nerve conduction studies show that compound muscle action potentials (CMAP) are decreased and distal motor latencies are often prolonged. Both sensory nerve action potentials (SNAP) and sensory evoked potential are reduced or absent.[10, 11, 13] Although no curative therapy for SBMA has been identified, androgen deprivation with a luteinizing hormone-releasing hormone (LHRH) analog has been shown to suppress the decline of both motor function and swallowing in SBMA patients.[14-16]

Although the histopathological hallmark of this disease is an extensive loss of lower motor neurons in the spinal cord and brain stem,[2, 17, 18] there are few studies on clinical measurements of neuronal loss in these patients.[11, 13] Motor unit number estimation (MUNE), which provides a quantitative estimate of the number of motor units innervating a muscle using electrophysiological methods, has been applied to various neurodegenerative diseases affecting lower motor neurons, such as amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), and Charcot-Marie-Tooth disease (CMT).[19-23] MUNE and muscular strength have been shown to correlate in patients with motor neuron disease.[24] There are, however, few reports on MUNE in SBMA.[25] The aim of this study is to count the number of motor units in SBMA patients, as well as to examine whether MUNE is a potential biomarker that reflects the pathophysiology and progression of motor neuron degeneration in this disease.

Materials and methods

Patients

A total of 52 patients with a diagnosis of SBMA confirmed by genetic analysis and 13 age-matched normal control subjects were included in this study. The normal controls were recruited from Nagoya University Hospital; we confirmed by clinical examination that they did not have any neuromuscular disease. The data were collected between March 2005 and August 2008. After the MUNE evaluation, 41 of 52 patients were enrolled in a clinical trial of an LHRH analog. Follow-up data of the remaining 11 patients were collected approximately one year after the initial examination. In addition to MUNE, we also analyzed motor function, disease duration and CAG repeat size in the *AR* gene. We defined the onset of disease as the time when muscular weakness began, but not when tremor of the fingers appeared. As a functional assessment, we applied the Limb Norris score, Norris Bulbar score, and ALS functional rating scale-revised (ALSFRS-R), which are aimed at evaluating motor function in patients with amyotrophic lateral sclerosis (ALS).[26, 27] Grip power of both the right and left hands was measured as described previously.[28]

MUNE

We examined MUNE using multiple point stimulation.[29, 30] In each patient, the maximum CMAPs and single motor unit potentials (SMUPs) were recorded in the right hypothenar

muscles by stimulating the ulnar nerve. The stimulating point was moved and 8-10 single motor unit potentials were obtained. Mean SMUPs were acquired with a Neuropack MEB-2200 (Nihon Kohden, Tokyo, Japan). The onsets of SMUPs were aligned to obtain averaged waveforms. To calculate MUNE, the amplitude and area of the negative portion of the CMAP were divided by the respective values of the averaged SMUPs. To assess reliability, the evaluation of MUNE was repeated in 8 patients within one week after the initial test. All evaluations of MUNE were performed by the same examiner (K.S.) throughout the period of this study in order to exclude the influence of inter-rater variability.[31]

Genetic analysis

Genomic DNA was extracted from the peripheral blood of SBMA patients using conventional techniques.[5] PCR amplification of the CAG repeat in the *AR* gene was performed using a fluorescein-labeled forward primer (5'-TCCAGAATCTGTTCCAGAGCGTGC-3') and a non-labeled reverse primer (5'-TGGCCTCGCTCAGGATGTCTTTAAG-3'). Detailed PCR methods have been described previously.[5] Aliquots of PCR products were combined with loading dye and separated by electrophoresis with an autoread sequencer (SQ-5500, Hitachi Electronics Engineering, Tokyo, Japan). The size of the CAG repeat was analyzed using Fraglys software version 2.2 (Hitachi Electronics Engineering) by comparison to co-electrophoresed PCR standards with known repeat sizes. The CAG repeat size of the PCR

standard was determined by direct sequence as described previously.[7]

Data analysis

Quantitative data are presented as mean ± SD. Statistical comparisons were performed using the Student's *t*-test. Correlations among the parameters were analyzed using Pearson's correlation coefficient. *p* values less than 0.05 were considered to indicate significance. Calculations were performed using the statistical software package SPSS 14.0J (SPSS Japan Inc., Tokyo, Japan).

Results

Clinical and genetic backgrounds of SBMA patients

The clinical details of SBMA patients studied are shown in table 1. All of the patients studied were of Japanese nationality. There was no significant difference between the median CAG repeat length in the present study and those reported previously in SBMA patients.[4-6, 16] All patients were ambulatory with or without aid, and none were bed-ridden. The mean Limb Norris score, Norris Bulbar score, and ALSFRS-R also suggested that the ADL of patients in this study was not severely impaired.

Reliability of MUNE

Pearson's correlation coefficients of test-retest values were 0.94 ($p = 0.001$) for MUNE values calculated by the amplitude of the CMAP, and 0.76 ($p = 0.03$) for those calculated by the area of the negative portion of the CMAP. The intraclass correlation coefficient was also higher in MUNE values calculated by amplitude (0.91, $p = 0.003$) than those calculated by area (0.84, $p = 0.01$). These observations suggest that the reliability of MUNE was excellent in this study, especially when calculated using the amplitude method.

MUNE in SBMA patients

MUNE values calculated by both the amplitude and the area methods were significantly lower in the SBMA patients than those in normal controls (table 1), and were similar to a previous report.[25] SBMA patients showed lower maximum CMAP than normal controls, whereas the SMUP of SBMA patients were higher than those of normal controls (table 1). In the majority of SBMA patients, MUNE values were less than the mean – 1 SD of normal controls (supplemental table 1). There was a strong correlation between the MUNE value calculated by the amplitude and that calculated by the area ($r = 0.81, p < 0.001$).

A significant correlation was observed between the MUNE values calculated by the amplitude and the disease duration (table 2, fig. 1A). MUNE values did not show a significant correlation with the age at examination, CAG repeat size, or motor functional scales. The MUNE values calculated by the amplitude were significantly correlated with grip

power in the ipsilateral hand (table 2, fig. 1B), but not with that of the contralateral hand (table 2, fig. 1C).

Natural history of MUNE

In a group of 11 SBMA patients, the MUNE values were significantly decreased 55.5 ± 4.3 (49 - 64) weeks after the initial evaluation (table 3, fig. 2). In contrast, CMAP, SMUP, motor functional scales, and grip power failed to show significant differences between the two examinations (table 3A). We confirmed that there were no differences between the demographics of the 11 patients and the other cases who did not undergo the follow-up study (table 3B).

Discussion

In motor neuron diseases such as SBMA, loss of motor neurons is the most fundamental pathological finding. It is thus important to measure the number of remaining motor neurons to evaluate the status of motor neuron degeneration. The present study described an extensive decrement of MUNE and a remarkable increase in SMUP in patients with SBMA.

These results suggest that both motor neuron loss and axonal reinnervation, which are often observed in the chronic stage of motor neuron diseases, influence MUNE and SMUP in SBMA. Muscle biopsies from patients with lower motor neuron disorders have showed a

high terminal innervation ratio caused by the nodal sprouting.[32] MUNE in motor neuron diseases has been shown to decrease generally,[25, 33-36] while the amplitude of SMUP varies according to specific disease properties. For example, SMUP has been reported to be elevated in SMA, which is characterized by a chronic degeneration of motor neurons similar to SBMA.[33] Several reports have described a significant increase of SMUP in ALS patients,[34, 35] whereas no obvious difference has been detected in other studies.[36] Given the slow progression of SBMA, axonal sprouting induced by long-standing neurodegeneration is likely to be the basis for SMUP elevation in these patients.

In the present study, MUNE values calculated by amplitude showed a significant correlation with the duration from the onset of muscle weakness. Based on our findings, we calculated the hypothetical MUNE at the time of onset using the regression line (fig. 1A). As a result, the MUNE at onset was estimated to be 97.2, which is considerably less than that of the normal controls. This calculation suggests that the loss of motor neurons had substantially progressed by the time clinical symptoms, such as muscular weakness, appeared in SBMA patients. A similar speculation has been reported in a MUNE analysis of superoxide dismutase 1 (SOD1) mutation carriers of familial ALS,[37] and in a histopathological study of autopsied ALS cases.[38]

Our results also showed that MUNE values calculated by the amplitude of the CMAP were closely correlated with ipsilateral, but not with the contralateral, grip power. MUNE of

motor neuron diseases, including ALS, has also been shown to be correlated with grip power in a previous study.[24] These correlations are likely due to the anatomical proximity in the spine of the hypothenar muscle examined in our MUNE study and the musculus flexor digitorum superficialis or the musculus flexor digitorum profundus that generate grip power. Collectively, our results suggest that the MUNE value reflects motor neuron loss in the cervical spine. Given that asymmetric muscle weakness is often seen in motor neuron diseases including SBMA, the fact that MUNE values correlate with the grip power of the ipsilateral hand but not the contralateral hand is likely to reflect the lateralized motor neuron loss within the spinal cord.[39, 40] In a one-year follow-up study, we found a significant decrease in MUNE without a significant change in SMUP. These findings suggest that axonal sprouting is incapable of compensating for the degeneration of motor neurons over a short period such as one year. Although the decline of MUNE in SBMA was slower than that measured in ALS,[41] it was similar to that in SMA type 2.[22] Given the slow progression of muscle weakness in SBMA, our results also suggest that MUNE is an electrophysiological parameter that can quantify disease progression in these patients.

Although the present study indicates that MUNE in SBMA patients fulfills the requirements for a biomarker,[42] caveats about this methodology have been reported.[20, 23, 43] A potential drawback of MUNE is the fact that the method and the examiner influence the measurements. To overcome this problem, various methods have been developed to measure

MUNE values: the incremental technique, spike-triggered averaging, multiple point stimulation, and statistical analysis.[20, 23, 29] Among these techniques, we employed the multiple point stimulation method, because it is non-invasive and as reliable as the other methods [20, 23, 29]. The high reproducibility of MUNE in the present study suggests that a fixed method and examiner appear to improve the usefulness of this measurement.

In conclusion, the present study shows a reduced MUNE in SBMA patients and a close relation between MUNE and motor function. In addition, this study reveals a significant decrease in MUNE within a relatively short time period. These findings suggest that MUNE is an electrophysiological biomarker that may be used to evaluate the severity and progression of motor neuron degeneration in SBMA.

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Competing interests: None.

Ethics approval:

The ethics committee of Nagoya University Graduate School of Medicine approved the study, and all SBMA and control subjects gave their written informed consent.

Figure legends

Figure 1 Relationship between MUNE and clinical backgrounds in SBMA patients. (A) Correlation between MUNE and disease duration. MUNE values calculated by the amplitude gradually deceased along with disease progression. (B-C) Correlation between MUNE and grip power in SBMA patients. MUNE values calculated by the amplitude showed a significant correlation with the ipsilateral, but not contralateral, grip power.

Figure 2 Individual cases of the changes in MUNE. MUNE values calculated by both the amplitude (A) and the area of the negative portion (B) were significantly decreased at the one-year follow-up examination in SBMA patients.

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